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STUDIES OF COLLAGEN *

I. THE PRODUCTION OF COLLAGEN IN VITRO UNDER VARIABLE EXPERIMENTAL CONDITIONS

GEORGE HASS, M.D., AND FRANCIS MCDONALD, M.D.

(From the Department of Pathology, Harvard Medical School, Boston, Mass.)

INTRODUCTION

A satisfactory definition of collagen has never been devised. Morphologists have gained a common concept of the meaning of the term through the medium of descriptive terminology. They regard collagen as a homogeneous or fibrillary intercellular protein which serves as the principal organic matrix of white fibrous connective tissue, bone and cartilage. They distinguish collagen from other intercellular materials by its staining reactions, microscopic structure, and other more or less empirical properties. Chemists regard collagen as a protein which yields gelatin upon partial hydrolytic degradation. It is clear that neither concept permits the designation of collagen as a chemical entity in any one species or among the several species in which it has been described.

The source of collagen is unknown. In processes of growth and repair it appears in the intercellular medium only in the presence of fibroblasts and is initially resolved as a morphological entity in the immediate vicinity of these cells. Consequently it is generally believed that fibroblasts perform an indispensable rôle in collagen formation. An unrestricted acceptance of this belief is tempered by the observations that collagen may be deposited, maintained at a steady state, or resorbed in the presence of these cells.

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Wolbach and collaborators [1, 2, 3] have penetrated deeply into one fundamental aspect of unknown mechanisms which regulate collagen production. They defined absolute scorbutus as an abnormal bodily state in which all deposition of collagen and other intercellular materials ceased. If the state of absolute scorbutus was interrupted by supplying the experimental animal with adequate amounts of ascorbic acid, collagen deposition was promptly resumed. Consequently the deduction was made that ascorbic acid is implicated in the mechanism of collagen formation. Beyond this the problem has been refractory to further fundamental analysis.

Maximow,[4] and later Maximow and Bloom,[5] proved that collagen is deposited around fibroblasts during prolonged cultivation of these cells *in vitro*. This method of investigation, despite its many disadvantages, provides a simplified system within which the influence of certain variables on collagen production can be studied to an extent impossible in the intact animal. Therefore, it seemed appropriate to employ this technic with three principal purposes in mind: (1) to standardize a method for assaying the capacity of cells and tissue fluids to promote collagen deposition, maintenance or resorption; (2) to study the influence of certain variables on the mechanisms which regulate the state of collagen in isolated systems of cells and tissue fluids; and (3) to develop a first technical step in more complicated experimental devices by which the cultures of cells may be brought into more intimate contact with the living tissue of the intact animal. The studies to be reported here are concerned principally with standardization of the method of assay and an inquiry into the influence of a few variables on the state of collagen in tissue cultures.

METHODS

The technic conformed to the principles of plasma clot cultivation of cells *in vitro*. Tissues from healthy guinea pigs and rats were used. In preparation of tissue for explantation, lung and spleen were isolated. Strips of tissue about 1–2 mm. in breadth were excised by linear incisions parallel to the acutely angled borders of these organs. The thin strips were then divided transversely at 1 mm. intervals. Explants so obtained were of relatively uniform size, shape and composition. Except when other-

wise stipulated, fresh homologous heparinized plasma was used in preparation of the medium. The clotting principle and growth-promoting factor were obtained by extracting fresh homologous crushed splenic tissue (circa 500 mg.) with 5 cc. of Tyrode's solution. Explants were placed in cylindrical, flat bottomed glass micro dishes (10 mm. in diameter and 5 mm. in height). Equal volumes of plasma and splenic extracts were then dropped into each dish in sufficient quantity (circa 0.1 cc.) just to immerse the explant. Routinely six simultaneous cultures comprised a unit set of each control experiment and an equal number of simultaneous explants were subjected to the variation under investigation. The unit sets were then placed in inverted petri dishes. After sealing the petri dishes with a paraffin-vaseline mixture, they were placed in the incubator at 36–38° C. Every 4 or 5 days the clot overlying each tissue explant was washed with a drop of fresh homologous heparinized plasma and splenic extract. Explants, therefore, were permitted to grow continuously *in situ* throughout the entire period of cultivation (6–40 days). At the termination of a desired period of cultivation the clot with the included culture was mechanically detached from the surface of the micro dish. Two representative cultures in each unit set of six cultures were selected and while still embedded in the plasma clot were fixed in Zenker's fluid. Serial sections were cut from paraffin blocks and stained with Mallory's aniline blue collagen stain.

Guinea pigs were maintained on an adequate laboratory ration, greens being supplied two or three times each week. Critical experiments were never done unless the animals had received 10–15 mg. of crystalline ascorbic acid orally each day for a period of 2–3 weeks prior to utilization of their tissues.

White rats used in the experiments were maintained on the usual laboratory diet.

For purposes of evaluating the influence of the age of animals on collagen production by their tissues, healthy animals of variable weights were used. Guinea pigs weighed within the following limits in the two series of experiments which were done: 100–150 gm., 200–250 gm., 300–400 gm., 400–500 gm. and 600–800 gm. In two similar experimental series the rats weighed within the following limits: 50–75 gm., 100–125 gm., 125–175 gm. and 200–300 gm. Cultures of spleen and lungs were maintained for

10–15 days *in vitro*. Mediums for replenishing cultures were obtained from animals of about the same weight as the animal whose tissues were explanted.

The temperature variable was studied by incubating four series of cultures of rat and guinea pig spleen within the temperature range of 34–41° C. In each series there were three unit sets of six cultures; one at 34–36° C., the second at 36–38° C., and the third at 38–41° C. Cultures in each series were explanted simultaneously and manipulated in as nearly an identical way as possible during the 9–12 day period of incubation.

Two methods were available for evaluating the relationship between the length of the period of cultivation and the amount of collagen deposited. First, a large number of cultures which either served as routine controls or failed to respond to variations under investigation were compared. Second, four series of experiments were conducted in which cultures explanted at the same time and treated similarly during the period of cultivation were fixed at appropriate intervals, such as 6, 12, 18, 24, 30 and 36 days after explantation. In this way the statistical results obtained by tabulating random cultures could be checked against results of controlled experiments in which, except for the length of the period of cultivation, the cultures had a similar history.

It was not possible to evaluate properly the rate of fibroblastic proliferation. However, under normal conditions the state of a culture at the termination of an experiment served as a crude index of the average rate of growth. This was estimated with fair accuracy by microscopic study of the whole of each culture in serial sections.

Another important variable which was often beyond satisfactory control or evaluation was the state of relative viability of cells. The evidences of extent of new growth, cellular division and cellular disintegration principally determined estimations of local or general viability of fibroblasts. This method had numerous disadvantages. Studies of oxygen consumption might have improved the interpretations, but such additional data could only have furnished information concerning average values for entire cultures and would not have aided in the interpretation of viability of particular groups of cells. Such isolated clusters of cells were often the only cells in an entire culture which were pertinent

to the problem under investigation. The morphological method, therefore, despite its disadvantages, seemed to possess the most advantages.

The investigation of the relation of the hydrogen ion concentration of the medium to collagen deposition was made as follows: The total range of pH subjected to study was from 6.6–8.0. The values were measured colorimetrically by the use of indicator dyes and standards. Ascorbic acid, acetic acid, sodium bicarbonate and potassium hydroxide in dilute solutions were used for regulation of the pH values. No accurate maintenance of a particular pH was obtained in any culture of this series. This was true, because even though initial pH values were adjusted within specified limits, these values shifted slightly during the period of cultivation in an unpredictable manner. The methods provided a crude index of average pH values over a stated period, maintained within the stipulated limits by appropriate addition of acid or base. The limits for controlled sets of cultures were as follows: pH 6.6–7.2, pH 7.2–7.7 and pH 7.7–8.0.

Experiments were conducted in two ways. The first method consisted of an initial adjustment of the pH value of the medium at the time of explantation followed by daily washing of the cultures with solutions having the desired pH value. By the second method cultures were grown under normal conditions for 8–10 days. One unit set was maintained under standard normal conditions. A second unit set was washed daily with solutions having a pH below that of the normal control set. A third unit set was similarly treated with solutions having a pH above that of the normal control set. This second method was more satisfactory than the first because the shifts of pH values were introduced at a time when collagen deposition was actively progressing within all cultures of the three comparable unit sets.

Three lines of procedure were followed in attempts to establish a relationship between the amount of titratable ascorbic acid in the medium and the amount of new collagen which appeared in the cultures.

The first procedure involved comparative studies of collagen deposition around cells derived from rats and guinea pigs. Guinea pigs are highly susceptible to scorbutus because they depend exclusively on an extrinsic source of ascorbic acid. In any lot of

guinea pigs the values of plasma ascorbic acid often vary within rather wide ranges. Rats, on the contrary, are resistant to scorbutus. They possess intrinsic mechanisms capable of synthesizing the vitamin and maintaining fairly constant values of ascorbic acid in their tissues. Therefore, the choice of animals provided control of an initial variable in one species even though it could not be accurately controlled in the second species. In continuing this comparative study, homologous and heterologous explants and mediums were permuted so as to provide nearly identical mediums for the support of growth of rat and guinea pig fibroblasts.

The principle of the second method was to provide as low a level of ascorbic acid in the culture mediums as was feasible without resorting to a choice of scorbutic animals for materials. The preparation of splenic extract in high dilutions and the dilution of plasma to twice its volume with splenic extract permitted, theoretically, and by indophenol colorometric titration, initial ascorbic acid values in the medium which averaged less than one-half normal plasma values. The natural instability of ascorbic acid under the experimental conditions was a further aid in realizing low levels in the medium during the period of cultivation. Fluid was expressed from the clots at 1, 2 and 3 day intervals after explantation. The indophenol titration values after the 1st day of incubation were so low as to be negligible or hardly beyond the range of error of the method of titration. The addition of diluted plasma at 3–4 day intervals in amounts never exceeding 10 per cent of the volume of the medium surrounding the explant did not materially influence the average low or negligible value of ascorbic acid in the medium during the period of cultivation. Therefore, routinely, fibroblasts were in a medium which contained very small amounts of ascorbic acid throughout almost the entire period of each experiment.

In order to reduce the values of ascorbic acid to still lower levels, $Cucl_2$ was added to the medium in which explants were embedded. The toxicity of $Cucl_2$ was so great that concentrations exceeding $10^{-5} - 10^{-6}$ gm./cc. of medium could not be used. Theoretically such concentrations as were employed should promote the rate of degradation of ascorbic acid.[6] No indophenol titrations were done on mediums to which copper ions were added.

A third set of experiments was designed to elevate the ascorbic

acid values of the culture medium. Crystalline ascorbic acid which proved to be sterile in sealed ampoules, in which it was available, was added directly to the medium just before utilization of the fluid in preparing the culture or before washing the explants *in situ*. The tissues tolerated circa 0.5 mg./cc. of ascorbic acid in the plasma-extract medium without additional buffering. This concentration was set as a standard maximum in the initial medium surrounding the cultures. Subsequent to explantation the cultures were washed daily with a medium similar to that used for explantation. In a selected series of cultures at various intervals indophenol titrations of the fluid expressed from the fibrin clots proved that this method maintained levels of ascorbic acid which averaged well above those of normal plasma.

A second method was employed in which the cultures were permitted to proliferate under routine conditions for 8–12 days. Each day thereafter, for 4–6 days, the surface of each explant was washed with the ascorbic acid-plasma-extract medium. These experiments were more satisfactory than those described above.

In the preceding two methods the pH values of the medium were consistently below the normal physiological range.

The third method consisted in daily addition to unit sets of cultures of an ascorbic acid-plasma-extract medium adjusted to a pH of 7.4–7.7. All cultures were maintained under the specified conditions for periods varying from 9–18 days.

Results

Cells which grew from the margins of each explant were predominantly fusiform or stellate mesenchymal cells. They were arranged in sheets, masses or radiating cords as primarily determined by the physical conditions of surfaces on which they had grown. For want of more precise methods of definition these immature cells were regarded as fibroblasts.

The new deposits of collagen were never as voluminous as those which are routinely observed in normal cicatrization of extensive wounds. The deposits invariably appeared in the immediate vicinity of the new growth of cells. The new collagen was always most abundant near the margins of the initial explant, especially where splenic pulp and capsular or pleural preformed collagen had been transected. It decreased in quantity

in direct proportion to the distance from these margins and rarely appeared around cells which had penetrated deeply into the fibrin clot. Isolated cells, particularly those which were far out in the clot and which had no direct continuity with projecting radial cords of cells, were never surrounded by collagen. The new deposits were usually fibrillary in structure and lay either between fibroblasts or between the cell boundary of a fibroblast and the adjacent fibrin of the clot.

Three fibrillary structures which did not select the definitive stain for demonstrating collagen were recognized among the new deposits of collagen.

The first type of non-collagenous fibril was of the fibroglia variety. These fibrils which stained red by Mallory's aniline blue collagen stain conformed to the contour and protoplasmic distribution of fibroblasts. Although they were often intimately associated in space with a fibrillar collagen, there were no good reasons for considering them as precursors of collagenous fibrils.

The second type of fibril was unstained in these preparations. These fibrillar structures were wavy and highly refractile. They were located in the immediate vicinity of fibroblasts and conformed to the spatial distribution of collagen. However, they were frequently numerous in the absence of collagen or in those cultures from which new deposits of collagen were undergoing resorption.

The third type of fibril was fibrinoid in character. These fibrils had no constant relationship to cells. They appeared primarily along lines of stress and frequently extended from the distal cells of the new growth for distances of 2–3 mm. to the margin of the clot. These condensed bundles of fibrin had no apparent relationship to collagen. However, when the fibrin meshwork was distorted so as to conform locally to the contour of fibroblasts, the resulting fibrinoid fibrils occasionally resembled the adjacent collagenous fibrils. The similarities in structure were rarely enhanced by an irregular bluish coloration, so that in improperly stained preparations these fibrils were easily confused with collagenous fibrils.

The quantity of collagen which appeared around fibroblasts of splenic origin was much greater than that which was deposited around fibroblasts of pulmonary origin (see Chart 2). Despite

numerous attempts, appropriate conditions for obtaining consistently good deposits of collagen around pulmonary fibroblasts were not established. The new collagen, if present at all, was

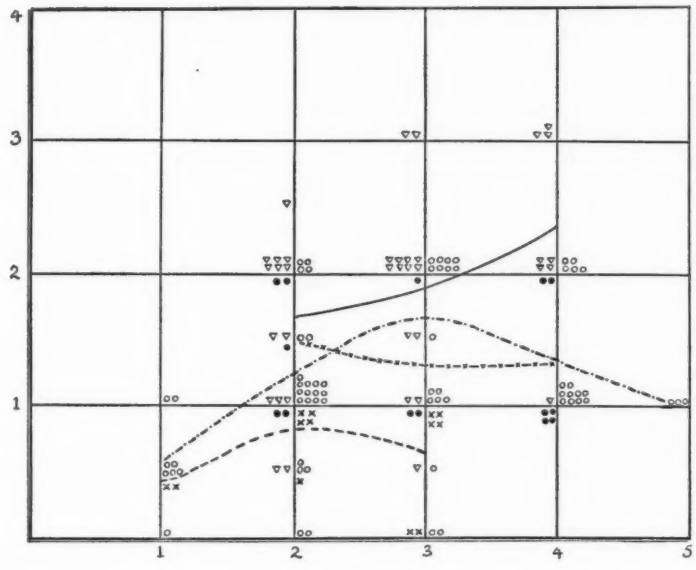

CHART 1

Ordinate = amount of new collagen deposited.
Abscissa = amount of fibroblastic proliferation.
Parameter = age of culture.
x = 2 cultures 6–8 days of age.
o = 2 cultures 8–12 days of age.
v = 2 cultures 12–18 days of age.
· = 2 cultures 18–32 days of age.
– – – – 6–8 day cultures.
–·–·– 8–12 day cultures.
——— 12–18 day cultures.
–x–x– 18–32 day cultures.

usually so meager in amount that explants of lung tissue were finally dispensed with as objects of study.

Cultures of rat tissue frequently contained more collagen than comparable cultures of guinea pig tissue. This was not a constant

finding and was not of sufficient magnitude to excite much interest. Attempts to investigate the meaning of the slight difference by growing rat tissue in guinea pig mediums and guinea pig tissue in rat mediums were unsuccessful. Although rat tissue grew fairly

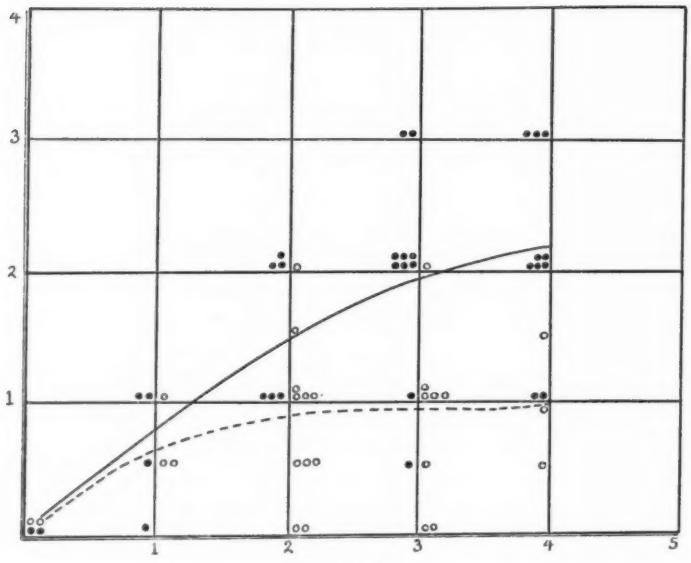

CHART 2

Ordinate = amount of new collagen.
Abscissa = amount of new growth of cells.
o = 2 cultures of lung tissue 9–16 days old.
• = 2 cultures of splenic tissue 9–16 days old.
– – – collagen appearing in lung cultures with respect to amount of fibroblastic proliferation.
——— collagen appearing in splenic cultures with respect to amount of fibroblastic proliferation.

well in guinea pig plasma and tissue extract, guinea pig tissue failed to proliferate to a desirable extent in rat plasma and extract. In either instance collagen deposition was wholly unsatisfactory, due primarily to restricted growth and diminished viability of cells in heterologous mediums.

The age of animals, as indicated by weights of the healthy

animals used in these experiments, could not be consistently correlated with the quantity of collagen which was deposited. In general, the best results were obtained by using animals of medium weight.

The temperature of incubation within the limits 34–41° C. was not a factor of first importance in the regulation of collagen production. Growth of cells was satisfactory within this temperature range. A comparison of cultures grown under controlled conditions, except for the temperature variation, revealed no difference in the amounts of newly deposited collagen, provided that the cultures were similar with respect to extent of growth and viability of cells.

The duration of cultivation of tissues proved to be a controllable variable of first importance. The results of these experiments are shown in Chart 1. In general, if a satisfactory growth of fibroblasts occurred, the amount of collagen which was deposited increased with time until the cultures were about 14–16 days of age. At this time, even though a culture continued to grow and cells retained their viability, collagen production ceased. A prolongation of the period of cultivation was usually accompanied by a resorption of collagen around fibroblasts. These facts were most clearly demonstrated in the several controlled series of cultures which were explanted simultaneously and fixed in Zenker's fluid at regular intervals of time between 6 and 40 days.

The extent of growth of the cultures and the viability of cells, as evaluated by microscopic studies, likewise proved to be important variables (see Chart 1). If a culture failed to grow, no new collagen was deposited even though cells within the explant remained viable. A voluminous growth of fibroblasts, however, was not always accompanied by collagen deposition. This was especially true in young cultures which grew with abnormal rapidity. In older cultures there was a better correlation between the extent of growth and collagen deposition, especially in the 12–18 day period, in which the arbitrary relationship between the estimated mass of new growth and collagen deposition was nearly linear (see Chart 1). Therefore, it became customary, after having studied many cultures of 12–18 days of age, to expect a certain amount of collagen to appear in the neighborhood of a certain number of fibroblasts. When this expectancy was not

fulfilled under normal conditions of cultivation the common morphological denominator, as a rule, was evidence of degeneration of fibroblasts. The cells in these instances were frequently vacuolated and swollen, or attenuated and poorly defined. Occasionally they contained small granules or globules of material which stained blue by Mallory's method with somewhat greater intensity than collagen. The data definitely indicated that resorption of new collagen and cessation of collagen production accompanied morphological evidence of early fibroblastic degeneration.

Another variable which influenced the deposition and resorption of collagen was the pH of the medium. A depression of the pH of the medium to lower limits which permitted continued growth and viability of fibroblasts was usually correlated with a cessation of collagen deposition and evidence of resorption of new collagen. This occurred irrespective of whether the pH was lowered by the use of ascorbic acid or acetic acid. The special control cultures to which equivalent amounts of these acids were added, plus sufficient base to bring the pH of the medium to within normal or still more alkaline ranges, were indistinguishable from the cultures which were always run as routine controls.

No satisfactory relationship could be established between the ascorbic acid content of the medium and collagen production. It was proved that under routine conditions of cultivation the quantity of ascorbic acid in the medium rapidly reached very low values. Collagen was deposited under these circumstances at a slow but steady rate. The addition of $Cucl_2$, which theoretically should increase the rate of disappearance of ascorbic acid, had no effect on collagen production. The daily addition of freshly prepared solutions of ascorbic acid to growing cultures did not increase the quantity of collagen which was deposited. On the contrary, if precautions were not taken to adjust the pH to within nearly normal ranges, the deposition of collagen was retarded and its resorption was enhanced. Cultures in which the pH of the medium was properly adjusted to counteract the acidity of ascorbic acid were indistinguishable in so far as the quantity of collagen was concerned from the control routine cultures. These results were consistent irrespective of whether tissues were derived from guinea pigs or rats.

Discussion

Several investigators have studied the formation of fibrillar structures *in vitro*. Among them, Maximow and Bloom,[4,5] Baitsell [7,8] and Lewis [9] may be mentioned.

Maximow and Bloom studied collagen formation around rabbit fibroblasts in cultures which had been repeatedly transected and reimplanted. Homologous plasma clots and extracts of embryonic tissue were the essential components of the culture medium. Collagen appeared in their cultures in significant quantities after 35–40 days cultivation *in vitro*. This prolonged period coincides with the period of cessation of production and depletion of collagen in cultures manipulated according to the routine procedure as outlined in this report.

Lewis observed fibril formation by direct microscopic inspection of living cultures of chick embryonic tissue in a fibrin-free medium. Fibrils formed and fused into bundles which appeared to be quite independent of the cells. Although Lewis may have been observing an uninterrupted formation of fibrils as a normal sequence in the differentiation of the embryonic intercellular substrate, the descriptions and illustrations call to mind the appearance of fibroglia fibrils rather than collagenous fibrils.

The fibrous structures described by Baitsell arose in plasma clots containing frog tissues. They formed along lines of stress in consequence of a distortion and realignment of the fibrin meshwork. Frequently these fibrils extended radially from the margin of the explant to the periphery of the clot and possessed no intimate spatial association with cells. In his opinion the fibrillar strands added support to the thesis that fibrin is transformed directly into collagen.

In experiments described here partially organized embryonic tissue and embryonic tissue extracts were intentionally excluded as undesirable variables. Plasma and extracts from tissues of animals well on the way to maturity were used. Furthermore, in standardizing the assay it was necessary to select tissues with fairly uniform initial morphology as a source for fibroblasts and to refrain from disturbing the explant during the period of cultivation. Significant deviations from this procedure led to major difficulties in the interpretation of data.

There were numerous advantages in having the whole of an undisturbed culture for study from a morphological point of view. New deposits of collagen were frequently voluminous in the neighborhood of transected margins of preformed mature collagen of the explant. Various interpretations of this relationship might be given but no data could be brought forward to support exclusively any one of the possible speculations. It is pertinent to consider the formulation of experiments devised to throw some light on the possible conversion of mature collagen to a more soluble substance which is amenable to local transportation in the medium with subsequent reconversion to a less soluble form of the material.

Many investigators believe that lymphocytes have something to do with collagen deposition in the intact animal. These experiments show that splenic tissues routinely were much more favorable than pulmonary tissues for the study of collagen deposition. It might be inferred that the numerous lymphocytes in splenic explants were responsible for this difference. If the lymphocytes played an important rôle in the mechanism of collagen deposition, the data failed to disclose the nature of this rôle. It may be stated that collagen deposits were similar whether located around fibroblasts growing among masses of lymphocytes or at a considerable distance from these cells. Furthermore, the impression was gained that cultures which contained the largest amounts of new collagen usually showed the most advanced disintegration of lymphocytes. Fully viable lymphocytes, on the other hand, did not seem to contribute in any way to collagen production.

There was no convincing evidence to substantiate the opinion of a few investigators that fibrinoid fibrils are transformed directly into collagenous fibrils. Nondescript strands and membranes of condensed and aligned fibrin were encountered routinely in these cultures. They occurred, principally, as described by Baitsell, along lines of stress not only around fibroblasts but also at random, often extending radially from the outermost cells of the new growth to the margin of the clot. In general these structures were spatially unrelated to new deposits of collagen and were red rather than blue in properly stained sections. Collagen deposits adhered to the contour of fibroblasts and usually lay either between such cells or between the cell membrane and the

limiting fibrin. When pericellular fibrin through the operation of stresses and disruption of transverse trabeculae of the meshwork yielded fibrillar structures, a confusing picture occasionally was presented. These fibrils in some instances acquired an irregularly distributed pale blue coloration. They offered no confusion in the instances where fibroblasts grew into the fibrin-free substance of the explant on the supports of preformed splenic structure. Wherever collagen was deposited, irrespective of the presence of fibrinoid fibrils, it acquired the same optical properties and distribution. Therefore, in our judgment, the confusion in distinguishing fibrinoid from collagenous fibrils was, in these few instances, incidental either to the adsorption of collagen upon fibrinoid fibrils or to unsatisfactory differentiation of the stain.

Fibroglia fibrils were as conspicuous in collagen-free areas as in regions where collagen was present in large quantities. The early deposits of collagen were frequently aligned along these fibrils but there was no evidence that fibroglia was transformed directly into collagen.

Among the deposits of collagen there often were many delicate refractile unstained fibrils. It is probable that they belong to the class of reticular or precollagenous fibrils. Inasmuch as they were very numerous in cultures from which collagen had been partially resorbed, the conclusion was drawn that some of them, at least, represented collagenous fibers which had lost their affinity for the specific stain.

The experimental data were in accord with the thesis of Wolbach and others that collagen is a secretory product of fibroblasts. The observations failed to provide critical proof that this thesis is correct. Collagen appeared as a morphologically defined substance in the immediate vicinity of fully viable fibroblasts. It was not recognizable within the cytoplasm of these cells and no particular morphological feature of normal fibroblasts could be correlated with the presence or absence of new collagen. It was certain that new collagen did not appear in increasing quantities or persist for a significant interval of time around fibroblasts which showed early morphological evidences of diminished viability. These manifestations were in the nature of increasing vacuolization or granularity of the cytoplasm in the presence of the normal external form of the cell. They occurred not only in fibroblasts around which

collagen had been deposited, but also in similar cells far out in the clot where no collagen was ever deposited. For these reasons, even though intracytoplasmic granules often stained with the same intensity as collagen, they were unacceptable as representative of an accumulated intracellular product destined to appear as collagen after secretion into the extracellular environment.

Therefore, conclusions which bear upon morphological aspects of collagen deposition *in vitro* may be stated as follows: First, under appropriate conditions collagen appeared in an intercellular medium composed of adult tissue components. Second, new collagen was not deposited unless fibrocytes, present initially in the explants, underwent proliferation. Third, fibroblasts created by this cellular division were a necessary but not a sufficient condition for progressive continuous collagen deposition. Fourth, areas in which preformed collagen or splenic pulp had been transected were principal selective sites for collagen deposition. Fifth, collagen was deposited only in the immediate vicinity of fibroblasts and diminished in amount around these cells in proportion to their distance from the margins of explants. Sixth, if products derived from lymphocytes contributed at all to collagen deposition, nonviable rather than viable lymphocytes should be regarded as potentially favorable sources of such products. Seventh, fibrinoid or other fibrillar structures were not defined as precursors of collagenous fibrils. Eighth, cessation of collagen production and resorption of new deposits of collagen accompanied early morphological evidences of diminished viability of fibroblasts. Finally, the first detectable stage in the process of resorption of new collagen was a loss of the selective staining reaction of the fibrils.

The physiology of collagen deposition is a part of the general problem of the physiology of tissue differentiation, for collagen is not only the elementary substance of white connective tissue but is also the principal organic substrate of cartilaginous and osseous matrices. Conditions which favor the transformation of a collagenous ground substance into a fibrous, cartilaginous or bony matrix have been reasonably well defined from a morphological viewpoint. The nature of mechanisms which determine the transformations is not understood. Until the physiology of collagen deposition, maintenance and resorption is clarified, the more com-

plex mechanisms which govern its differentiation will probably remain obscure.

The age of the animal from which the tissues and mediums were obtained could not be positively demonstrated as an important factor in the regulation of collagen production *in vitro*. From this, it may be inferred that humoral influences which were governing normal growth of the intact animal had no effect on collagen production in the isolated system under investigation. It is to be remembered that normal healthy animals were employed in all experiments. Radical deviations from normal established standards are subjects for future study.

The experimental data indicated that rat and guinea pig fibroblasts, if directly engaged at all in collagen deposition, behaved similarly under comparable conditions *in vitro*. Rat fibroblasts, therefore, possess no intrinsic stable mechanism which permits them to engage in collagen production to an extent impossible for guinea pig fibroblasts in a similar isolated system.

Deposits of collagen around splenic explants were much more abundant than those around pulmonary explants. Variables which were subjected to controlled assay did not disclose any reason for the difference. It seemed that the determining factors lay within the sphere of the differential cellular composition of the two tissues. Before the meaning of this consistent observation can be expressed in terms of the differential components, experiments of a more specific nature than those reported here must be done.

It was not surprising to find that the temperature of incubation within the stipulated ranges, permitting satisfactory sustained growth, had no important influence on the quantitative aspects of collagen deposition. These data were desirable from a metabolic point of view and as checks on unreported experiments in which the temperature variable was beyond accurate control.

Collagen deposition, maintenance and resorption were influenced by three interdependent variables, namely, the numerical increment in fibroblasts, the duration of the period of cultivation, and the viability of the new growth of cells. As a rule the maximum number of fibroblasts in a culture was reached within 12–14 days after explantation. From this time, throughout the remaining period of cultivation, the number of fibroblasts increased very

slowly, remained at a stationary value, or decreased. In so far as collagen deposition was concerned, the number of fibroblasts in cultures 8 days or less in age was not a factor of great importance (see Chart 1). From 8 to 12 days the correlation between the number of fibroblasts and the amount of collagen deposited was significant up to a medium value of growth. Excessively active growth, however, did not ensure an increment in collagen production. After the growth had become more or less quiescent, that is to say after about 12 days, the amount of collagen became a linear function of the estimated mass of growth of an average culture. At times the deviations from this relationship were very great and wholly beyond analysis, but in general the correlation was valid in the 12–18 day period. A prolongation of the period of cultivation beyond 16 to 18 days was characterized by a cessation of collagen production or a partial resorption of collagen which had been deposited.

These data are in accord with the known sequences of normal wound healing in intact animals. In the repair of wounds the period of active fibroblastic proliferation is followed by a period of relatively slow cellular division during which time the voluminous deposits of collagen appear. Finally, as was true *in vitro*, collagen deposition ceases and resorption often ensues, irrespective of the presence of viable fibroblasts. The essential differences between collagen production as observed *in vitro* in the splenic cultures and the behavior of collagen in uncomplicated healing of wounds were principally quantitative and temporal. In other words, much less collagen was deposited *in vitro*, its rate of production was delayed, and the time over which it continued to appear was somewhat prolonged. The cessation of collagen production in wound healing is a puzzling matter but is no more puzzling than the analogous phenomenon *in vitro*. In each system there are, normally, numerous viable fibroblasts around which collagen deposition occurs within particular time intervals and then, for wholly obscure reasons, stops altogether.

The state of viability of a cell has been used in this discussion as a term for simplifying a potentially complex and variable situation. A cell may be non-viable with respect to one of its normal functions, and fully viable with respect to the accomplishment of any one of several other functions. For instance, if there were but one criterion for measurement of fibroblastic function, namely

the continuous deposition of collagen around that cell, it would be legitimate to consider the fibroblast as non-viable if the deposition of collagen ceased. The same should hold true for the other postulated functions of fibroblasts. Inasmuch as the physiological states which would determine a functional analysis of viability remain undefined in terms of functional systems, the cytological concepts of diminished viability formed the basis for evaluating the empirical state of cell viability in these experiments. Despite this particular method of reckoning viability, the data persist as a basis for a future classification of the viability of pluripotential cells in terms of the functions which they accomplish or fail to accomplish under different sets of circumstances.

If dependence was placed on the usual cytological criteria for evaluating the state of viability of fibroblasts, it was clear that diminished viability was positively correlated, not only with retardation and cessation of collagen deposition, but also with resorption of regional new deposits of collagen. The mature preformed collagen of the explants, on the contrary, was not visibly affected by degenerative changes within fibroblasts. These facts, in addition to other data, illustrate that the new deposits of collagen were susceptible to environmental changes which did not appreciably alter the structural integrity of the mature collagen. This supports the belief that collagen does not always possess in any species a constant composition generally inferred from morphological studies.

These conclusions were necessarily founded on the interpretation of non-specific data. In other words, the state of any culture was never known at any time except at the termination of an experiment. However, it was usual for cultures to grow uniformly for a period of time. At the end of this period it became customary from experience to associate a given number of fibroblasts with a moderately variable quantity of newly deposited collagen. Not infrequently focal areas of fibroblastic degeneration occurred in these cultures. In these areas, comparable as to mass of growth with adjacent regions of viable cells, collagen deposits remained in a steady state, were diminished or were absent. In this process of resorption of new-formed collagen in the neighborhood of degenerating fibroblasts, the first stage was a loss of characteristic staining affinities and the subsequent stage a disappearance of the fibrillar structure.

Intracytoplasmic globules and granules which stained deep blue by Mallory's method often characterized fibroblasts around which collagen was disappearing. These cytological features, which were considered as early evidences of degeneration of fibroblasts, were not, however, specifically related to the spatial distribution of collagen deposition or resorption. Suffice it to say that although collagen was never increased or wholly maintained around cells which displayed these changes, the morphological changes were not a necessary part of the observations in regions of cessation of collagen production or collagen resorption.

The pH of the medium, whether regulated within the given ranges throughout the period of cultivation or after satisfactory growth in normal mediums had been obtained, influenced the quantity of new collagen as measured at the termination of the period of cultivation. The depression of the pH to low levels was positively correlated, not only with retardation of collagen deposition but also with actual resorption of new deposits of collagen. This observation was made with equal frequency in cultures adjusted to low pH values by addition of either ascorbic acid or acetic acid. Inasmuch as fibroblasts were not very resistant to low pH values of the medium, the question of viability entered into the interpretation of the data. Even though there was a retention of viability, as judged by morphological standards, a lowering of the pH of the medium was accompanied by restricted deposition and accelerated resorption of new deposits of collagen. These data have a distinct bearing on the problem of collagen deposition and resorption in the intact animal.

The above considerations lead to a discussion of the influence of low and high values of ascorbic acid on collagen production. Although low ascorbic acid values have been constantly correlated with a cessation of collagen production in the intact animal, similar low values had no influence on collagen production in the isolated system under investigation. Furthermore, the elevation of negligible values to above normal plasma averages by daily addition of ascorbic acid to the medium did not increase collagen production. On the contrary, unless steps were taken to maintain the pH of the medium within reasonably normal ranges, a reverse effect was observed. Collagen deposition was retarded and the resorption of collagen was enhanced.

There are several ways of measuring the general bodily state known as scorbutus. One measurement is chemical and depends on the titration of reduced ascorbic acid in some tissue, preferably the blood plasma. The second method of measurement is morphological and depends on restricted deposition of intercellular materials such as collagen or dentine.[1, 2] These two methods, in so far as they apply to the intact animal, yield complementary results in experimental scorbutus and to a variable extent in clinical human scorbutus. Under the conditions of the isolated system composed of fibroblasts embedded in a tissue-extract-plasma medium the two methods of measuring scorbutus lead to a conflict. In so far as the deposition of collagen was concerned, the splenic tissues failed to reveal the scorbutic state predictable on the basis of the negligible quantities of ascorbic acid in the medium. Nor could the morphological measurements be influenced by varying the chemical component, ascorbic acid, within wide limits from zero to far above values ever attained in the intact animal. Pulmonary fibroblasts, on the other hand, behaved essentially as cells in a quasiscorbutic state under all circumstances.

The data indicate clearly that the state of tissues and fluids *in vitro* was not comparable in one or more respects with the state of similar materials in the disease known as absolute scorbutus. The lack of significant collagen production around pulmonary fibroblasts, the satisfactory deposits of collagen around splenic fibroblasts, and the equivalent deposition of collagen around cells derived from two species, one susceptible to scorbutus and the second refractory to scorbutus, lead to the idea that the generalized concept of scorbutus may be amenable to extension, especially in the realm of pathology. This extension would include the hypothesis that local scorbutus may occur in tissues or organs in the absence of those conditions which lead to generalized scorbutus; and second, the hypothesis that in generalized scorbutus a tissue or organ may fail to behave according to prediction because of its inherent capacity to resist the means by which the vitamin deficiency induces manifestations of disease.

Summary

Methods based on principles of tissue cultivation were standardized to provide an isolated system for studying the deposition,

maintenance and resorption of collagen *in vitro*. This system, which was free from embryonic components, was subjected to several controlled variations.

Pulmonary and splenic tissues obtained from normal rats and guinea pigs were explanted in mediums routinely composed of homologous clotted plasma and aqueous splenic extract. Cells were permitted to proliferate continuously for 6–40 days without interference incidental to transection or re-explantation. Cultures selected for study were cut in serial sections and the new deposits of collagen were defined by accepted staining reactions.

Normally collagen began to appear around fibroblasts of the new growth within 6–8 days after preparation of the cultures. It increased in amount until the explants were 14–16 days of age. From this time onward, despite continued slow multiplication and persistence of viable fibroblasts, the quantity of new collagen tended to diminish rather than to undergo progressive increment. The sequence was comparable with the usual behavior of collagen in the uncomplicated healing of wounds.

The amount of new collagen deposited was independent not only of the temperature of incubation within limits permitting fibroblastic proliferation, but also of the age and species of the animal whose tissues were used as a source of fibroblasts and culture mediums.

Although lymphocytes were not established as a specific spatial determinant of collagen deposition, much more collagen appeared around fibroblasts of splenic origin than cells of similar morphology which had proliferated from the margins of pulmonary tissues.

New collagen was most abundant around fibroblasts adjacent to the explant and its deposition was favored in the neighborhood of transected, mature preformed collagen or splenic pulp, especially if the cells of the pulp were degenerating.

Although fibroglia fibrils, fibrinoid fibrils and unstained fibrils of undetermined nature were often intimately associated with the new deposits of collagen, it was not apparent that collagenous fibrils were derived from these fibrillar structures.

Cessation of collagen production and resorption of pericellular deposits of new collagen invariably accompanied early morphological evidences of diminished viability of fibroblasts.

In the presence of viable fibroblasts a sufficient depression of the pH of the medium proved to be an effective means not only of restricting collagen deposition but also of enhancing the resorption of new collagen.

Mature preformed collagen did not respond to the influences which were so important in governing the persistence of new collagen. Therefore, it seemed justifiable to conclude that immature collagen differs from mature collagen in that it possesses properties which render it more susceptible to the action of certain environmental influences.

There was no specific correlation between the amount of collagen deposited and average low or high values of ascorbic acid in the medium if the viability of fibroblasts and the pH of the medium remained within normal limits. If the two principal criteria for evaluating scorbutus in the intact animal were applied to the isolated tissues it was evident that splenic explants were refractory to scorbutus, while the characteristic quasiscorbutic state of pulmonary explants was not alleviated by an abundant supply of ascorbic acid in the medium.

The observations were in accord with a proposed hypothesis that a deviation from a standard organized state may create conditions under which a tissue need not respond to a theoretical adequacy or deficiency of a vitamin in accordance with generalized predictions based on the behavior of the tissue in the standard reference state.

Finally, the data direct attention to the probability that some unknown factor in tissues, beyond those specifically dealt with here, is necessary before normal fibroblasts and normal mediums interact to yield collagen *in vitro*.

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STUDIES OF COLLAGEN *

II. Methods and Results of Implantation of Collagen-Forming Cultures in Granulation Tissue

George Hass, M.D.

(From the Department of Pathology, Harvard Medical School, Boston, Mass.)

Introduction

Previous studies have shown that under varied *in vitro* conditions cells and tissue fluids differ in their capacity to promote the deposition, maintenance and resorption of collagen.[1] Even under most ideal circumstances the amount of collagen which appeared around proliferating fibroblasts of tissue cultures was invariably less than that which is customarily observed in the normal repair of wounds. For this reason it seemed desirable to impose additional natural restrictions on the growing cultures by implanting them at the focal point of healing subcutaneous wounds. In doing this it seemed pertinent to separate the additional restrictions into two general classes by interposing permeable or semipermeable membranes between the healing tissues and fluids bathing the implanted cultures.

Technic

In order to achieve the stated objectives three experimental units were required: (1) cultures of proliferating fibroblasts; (2) a technic by which the growing cultures could be maintained in contact with traumatized healing tissues of the intact animal; and (3) reasonably uniform sites of implantation in experimental animals.

The *in vitro* technic for providing cultures of proliferating fibroblasts was identical with that described in a previous report.[1] Splenic and pulmonary tissues obtained from healthy rats and guinea pigs were cultivated continuously *in vitro* for periods ranging from 6–18 days. These cultures were available in small glass micro dishes (Fig. 9) which were divided into two unit sets, one for implantation and the second for control purposes.

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The apparatus which served to protect the cultures during their residence in areas of wound healing was composed of three types of stainless steel units and two types of membranes.

One type of steel unit was a cylinder for housing the glass culture dishes (Fig. 5). The cylinder had the following dimensions: total diameter 14 mm., internal diameter 12 mm., and height 5 mm. The wall of the cylinder was smooth internally and threaded externally.

The second type of unit was a cylindrical band with one right-angled flange (Figs. 1 and 2). The band was ribbed externally to provide a corrugated surface for traction and threaded internally to conform with the threads on the external surface of the cylinder. Each metal band possessed one right-angled flange, machined so as to fit the metal cross-sectional area of either end of the cylinder. The bands were of such height that when screwed tightly onto each end of the cylinder the apparatus had the appearance of a single unit, namely a hollow cylinder with two open ends, a smooth internal surface and a corrugated external surface (Fig. 11).

The third type of metal unit was similar to the cap described above. It differed in that the metal flange extended almost to the center of the cap (Fig. 6). At the center there was a hole 1 mm. in diameter. This led into an upright hollow cylinder (3 mm. in external diameter and 5 mm. in height) which was a part of the same block of steel as the cap (Fig. 6). This cylinder was threaded externally to accept a small metal cap which fitted securely over its distal end (Figs. 7 and 8).

For preparing membranes so that they would fit accurately a sharp steel cutting tool was made. With this hollow cylindrical tool small disks of cellophane having a diameter identical with the internal diameter of the ring-shaped caps were conveniently prepared from strips of cellophane such as are ordinarily used for dialyzing proteins (Fig. 3).

The routine preparation of the apparatus for implantation was as follows: Two ring-shaped caps were fitted with cellophane disks. One cap was screwed tightly over each end of the cylinder, thereby holding the cellophane windows firmly in place. A layer of glyptol was applied at the external angle of junction between the metal flange of each cap and the surface of each cellophane membrane (Fig. 4). After drying the glyptol in a stream of air one cap was

removed and the apparatus was placed in 30 per cent alcohol for 15–30 minutes. The parts were then washed thoroughly in several changes of sterile Tyrode's solution. A glass micro dish containing a growing tissue explant embedded in a plasma clot-splenic extract medium (Fig. 9) was placed in the cylinder. Sufficient homologous fresh serum was added to fill the micro dish (circa 0.2 cc.). The cap which had been removed was then slowly screwed into place. The growing culture embedded in fibrin clot and bathed in serum was thereby encased in a sterile metal cylinder possessing two semipermeable cellophane membranes (Fig. 10).

Two variations from the routine procedure were used. When freely permeable membranes were desired the cellophane disks were perforated in many places with the point of a needle. When a prolonged implantation period was desired one ring shaped cap with a cellophane window was dispensed with and a cap with the projecting hollow cylinder was screwed onto one end of the implantation receptacle in its place (Fig. 12).

The thoracic and axillary subcutaneous tissues of rats and guinea pigs were sites of implantation. After making a midsternal incision a cylinder was forced into each axillary region, coming to rest between the chest wall and the skin (Fig. 13). When it was desirable to leave the cylinder in place for more than 4 days one end of the cylinder was fitted with a cap possessing the upright columnar projection. A small incision of sufficient size to permit the column to project through the skin was made and a stay suture utilized to insure immobilization (Fig. 13). Following this the midsternal wound was sutured. More complete immobilization of the cylinder *in situ* was secured by a tight binder of adhesive tape. Sterile precautions were maintained throughout the procedure.

When two membranes were employed in the assembly of the implantation receptacle it was necessary to restrict the period of implantation to 4 days or less because irrespective of the degree of permeability of the membranes there was a progressive lysis of fibrin in which the tissue culture was embedded. This likewise occurred when only one cellophane window was used. In order to keep pace with fibrinolysis and prevent the cultures from floating free in the implantation receptacle it was necessary to introduce heparinized plasma at 4 day intervals. Whenever prolonged culti-

vation *in vivo* was desired the small metal cap covering the end of the hollow cylinder which projected through the skin was removed with sterile precautions. A pipette was introduced through the bore of the hollow cylinder into the implantation receptacle (Fig. 14). Excess fluid was aspirated and one or two drops of heparinized plasma were introduced into the micro dish which was properly oriented for this manipulation. A few minutes were required to ensure clotting of the fresh plasma overlying the tissue culture. The small metal cap was replaced and the animal was returned to its cage. By this method the lattice of fibrin which was necessary for proper continuous growth of the cultures was replenished and it was possible therefore to maintain cultures within the implantation receptacle in restricted contact with granulating tissues for as long as 18 days.

After conclusion of the desired period of tissue cultivation *in vivo* the implantation receptacle was excised from the granulation tissue. The metal caps were unscrewed. The culture was exposed, still embedded in fibrin clot, within the glass micro dish. The clot and enmeshed culture were detached from the inner surface of the culture dish and floated into Zenker's fluid. Control cultures of the same unit set were always maintained under standard *in vitro* conditions. They were fixed in Zenker's fluid, not only at the same time as the implanted cultures but also in selected instances at the time of implantation. Serial sections were made of each culture. Collagen was stained in the sections by Mallory's aniline blue method.

The two species from which tissues were obtained for cultivation, namely the rat and the guinea pig, the two species into which these tissues were implanted and the two types of membranes which were used afforded eight possible combinations. The number of experiments with each combination is shown in Table I.

The cultures, except in eight instances, were of splenic tissue. They were cultivated *in vitro* for 6–18 days prior to implantation. The majority of the implants were removed after 3–4 days of restricted contact with traumatized healing tissues. A few were maintained *in vivo* for 6–18 days. Only two cultures were maintained in the subcutaneous tissues for the maximum period of 18 days.

In order to be certain of abundant collagen production around

the implantation receptacles two series of experiments were performed in which four successive implantations were made in the same zone of active progressive cicatrization.

Results and Discussion

The metal cylinders were well tolerated by the tissues (Fig. 13). They were rapidly encapsulated by granulation tissue. In no instance was there loss of viability of the skin or serious secondary infection. When perforated membranes were used the granulation tissue usually grew through the perforations and spread as a gray,

Table I

Number of experiments	Source of explant	Site of implant	Type of membrane
24	Rat	Rat	Semipermeable
16	Rat	Guinea pig	Semipermeable
4	Rat	Rat	Permeable
2	Rat	Guinea pig	Permeable
20	Guinea pig	Guinea pig	Semipermeable
10	Guinea pig	Rat	Semipermeable
13	Guinea pig	Guinea pig	Permeable
2	Guinea pig	Rat	Permeable

thin cellular membrane along the inner surface of each cellophane window. Under these circumstances the fluid within the cylinder was frequently tinged with blood and slightly turbid. Therefore this technical device provided a means by which the tissue cultures were bathed, while still embedded in an homologous plasma clot, with homologous or heterologous fluids exuding from traumatized healing tissues.

When semipermeable membranes were used they formed an efficient barrier between the serum in the cylinder and the nondiffusible materials of the granulation tissue environment. As a rule the liquid-gas ratio within the cylinder at the time of implantation was about 1:1. During the residence of the implant in the subcutaneous environment this ratio increased. The increase may have been due to two factors: (1) the higher osmotic pressure of fluid within the cylinder, and (2) the absorption of a part of the gaseous phase. The liquid within the cylinder was invariably clear and of sufficient mobility and volume to ensure ready transport of diffusible soluble materials in each direction

across the semipermeable membranes. This device, therefore, provided a means by which cultures in homologous plasma clots were brought into contact with diffusible soluble compounds exuding from homologous or heterologous traumatized healing tissues.

At the time of implantation the tissue culture in the micro dish was securely embedded in a plasma clot which was homologous with respect to the growing cells. A distance of 1 mm. was the maximum permitted between the exposed surface of the plasma clot and the upper surface of the growing culture. This thin clot served as a partial barrier between the mobile liquid in the implantation receptacle and the cells of the culture. Normally this barrier was gradually removed through the action of some fibrinolytic mechanism so that the period within which cultures could be maintained without replenishing the clot did not exceed 5 days. This fibrinolytic mechanism seemed to be primarily independent of the nature of the membrane which was used. It was far in excess of the mild fibrinolysis which occurred *in vitro* in the control cultures. It might be argued that proteolytic ferments arising in the traumatized tissues traversed the membranes and were instrumental in causing lysis of the fibrin clot. If this were so, the data indicated that such ferments were capable of passing through a semipermeable membrane.

Inasmuch as new collagen was usually deposited between fibroblasts and the fibrin meshwork of the clot in which they were growing, fibrinolysis was a factor which had to be taken into consideration in evaluating the change in state of collagen in the implants.

Two other factors which were not encountered in the control cultures likewise had to be considered in the analysis of mechanisms governing the state of collagen in the implants. One of these factors centered about a change in morphology of fibroblasts. The other factor was a change in their spatial distribution.

The fibroblasts at the time of implantation presented the usual appearance of fibroblasts in tissue cultures. They were distributed around the margins of the explants in sheets or radially projecting columns. During the period of implantation there was a distinct tendency for the large cells to diminish in size and to acquire an accentuated fusiform or stellate shape. In other words, the imposed environment for some reason changed the fibroblasts from

"immature" to "more mature" types. These cells frequently possessed long tenuous processes and acquired a delicately vacuolated cytoplasm. As a rule the cells lost the regular radial polarization which was so characteristic of the control cultures. The shift of organization was in the direction of increasing symmetry of distribution of cells and their processes throughout the field of growth. There was no apparent explanation for this rearrangement. It may be that a redistribution of mobile components in the culture medium as a consequence of motion incidental to activity of the animal was important in disrupting the radial polarization of fibroblasts which characterized the stationary *in vitro* cultures.

Morphological evidences of diminished cell viability were fairly common, especially in those implants which had survived, unprotected by semipermeable membranes, in heterologous granulating tissues. When degenerative changes occurred they consisted of delicate cytoplasmic vacuolization, progressive atrophy and final attenuation of the cell body. This was in contrast to the swelling, granularity and coarse vacuolization which usually characterized degenerating cell bodies under *in vitro* circumstances.

All of these factors encountered *in vivo*, namely the action of fibrinolytic ferments, the alterations in cell morphology in the direction of maturity, the acquisition of new patterns of cellular arrangement and the peculiar type of cellular degeneration, made an evaluation of the state of collagen in terms of the control cultures more difficult than was expected. However, from a crude quantitative point of view the results of the experiments with respect to the amount of new collagen in the implants as compared with new collagen in control cultures are shown in Table II.

It is clear that implantation of cultures did not lead to uniform changes in the state of collagen. The most favorable permutations which resulted in an increased production of collagen were those in which cultures were implanted in homologous animals and separated from the healing tissues by semipermeable membranes. The results were almost equally as good when permeable membranes were used. The least favorable results were obtained when cultures were implanted in heterologous animals. In these instances the nature of the membranes had more significance. When they were freely permeable, cessation of collagen production and

resorption of new collagen were much more prominent than when they were semipermeable.

The data as tabulated are not a true indication of the quantitative aspects of the observations. In several instances the amount of collagen which appeared among cells of implanted cultures was greater than I have seen in any one of a large number of strictly

TABLE II

Number of experiments	Source of explant	Site of implantation	Type of membrane	Collagen in implants with respect to control cultures
24	Rat	Rat	Semipermeable	12 > Controls 6 = " 6 < "
16	Rat	Guinea pig	Semipermeable	2 > Controls 6 = " 8 < "
4	Rat	Rat	Permeable	1 > Controls 2 = " 1 < "
2	Rat	Guinea pig	Permeable	2 ⟨⟨ Controls
20	Guinea pig	Guinea pig	Semipermeable	6 > Controls 2 = " 12 < "
10	Guinea pig	Rat	Semipermeable	1 = Controls 9 < "
13	Guinea pig	Guinea pig	Permeable	4 > Controls 3 = " 6 < "
2	Guinea pig	Rat	Permeable	2 ⟨⟨ Controls

in vitro cultures. This unusual degree of collagen deposition occasionally was characterized by a diffuse, homogeneous blue-staining of the intercellular ground substance between rather widely dispersed fibroblasts. Over two hundred *in vitro* cultures were reviewed and none of them showed this distinctive peculiar type of collagen deposition. Unfortunately the abundant deposits of collagen were not constant and prolongation of the period of implantation or successive implantations in a mass of chronic granulation tissue did not consistently improve the results.

There was ample evidence that effective mechanisms were at work in removing new deposits of collagen from the implanted cultures. These mechanisms were apparently independent of the permeability of the membranes and were as refractory to segregation as the fibrinolytic mechanism which has been discussed.

The most pronounced cessation of collagen production and resorption of new collagen were obtained when implants were exposed to a relatively unobstructed flux of liquid emanating from heterologous granulating tissues. A glance at the tabulated data shows that these conditions were not necessary and that resorption of new collagen frequently occurred under circumstances theoretically ideal for its production. The resorption of collagen from the implants was commonly accompanied by morphological evidence of diminished viability of fibroblasts, but this was not always the case. The factors which were responsible for collagen resorption remain as obscure as the factors which promoted collagen deposition, fibrinolysis, cellular "maturation" and cellular rearrangement.

It may be concluded therefore that there were two antagonistic forces which influenced the state of collagen in the implants. One mechanism stimulated the production of collagen by fibroblasts of the implant. This was apparently effective only when tissues were implanted in homologous animals. A second mechanism promoted the resorption of new collagen from the implants. This was effective when tissues were implanted in either homologous or heterologous animals. The observations seemed to be a reflection of the preponderant effectiveness of one or the other mechanism. The functional components of either mechanism, if they emanated from the granulating healing tissues, could not exclusively be segregated by the interposition of semipermeable membranes between the fibroblasts of the assay culture and the granulating tissues.

Summary

Previous studies showed that collagen was deposited under appropriate conditions *in vitro* around fibroblasts proliferating from the margins of splenic and pulmonary tissues derived from normal rats and guinea pigs. The deposition began about 6 days after explantation and reached a maximum within 14–16 days.

In experiments reported here cultures during the active stage of collagen deposition *in vitro* were transferred to metal cylinders which possessed permeable or semipermeable cellophane membranes. The cultures housed in these receptacles were implanted in subcutaneous areas of trauma in rats and guinea pigs. The cultures were maintained in the areas of trauma for periods varying from 2–18 days. Replenishment of the clot of the culture medium surrounding the implant was required when a culture was left *in vivo* for more than 4 days. This was achieved without disturbing the continuity of the experiment. On completion of the desired period of implantation the cultures were removed from the granulation tissue environment and paraffin serial sections were prepared for microscopic study of collagen. Appropriate control cultures which were maintained under standardized *in vitro* conditions were available for comparison with the implants.

There were several interesting results. First, lysis of the fibrin clot gradually proceeded within the protective cylinder, irrespective of the interposition of a semipermeable or permeable membrane between the fluid bathing this clot and the granulating tissues. Second, cultures which had resided in the imposed subcutaneous environment displayed alterations in the general form and distribution of fibroblasts. Third, the state of collagen within the sphere of the proliferating fibroblasts of the implant was altered in an inconstant manner which was not amenable to accurate analysis. In general, conditions were most favorable for increased deposition of collagen when cultures were implanted in homologous animals and protected by semipermeable membranes. Conditions were least favorable for collagen deposition in the assay implants when tissues in homologous mediums were implanted in heterologous animals and separated from the granulating tissues by permeable membranes. Although excessive collagen production was quite impressive in a few implants, the overall effect was in the opposite direction, namely toward cessation of collagen production and resorption of collagen. It seemed reasonable, therefore, to conclude that fluids exuding from granulating tissues exerted two antagonistic influences on the assay cultures. One favored an increased deposition of collagen between the fibroblasts of the implants. The other retarded collagen deposition and led to resorption of newly formed collagen from the

implanted cultures. The observations were a record of the variable effectiveness of one or the other influence. Interposition of a semi-permeable membrane between fluids exuding from acute or chronic granulation tissue and the implanted assay cultures was of no value in segregating the effective components of either influence.

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DESCRIPTION OF PLATES

PLATE III

FIG. 1. Cylinder cap with flange up showing external corrugation.

FIG. 2. Cylinder cap with flange down showing internal threads.

FIG. 3. Semipermeable cellophane dialyzing membrane having the same diameter as the internal diameter of the cylinder cap.

FIG. 4. Cylinder cap; flange up; cellophane membrane sealed in place with glyptol; ready for assembly.

FIG. 5. Cylinder for housing Fig. 9, the glass micro dish containing tissue culture.

FIG. 6. Cylinder head with hollow metal threaded column.

FIG. 7. Metal cap to fit over threaded part of column in Fig. 6.

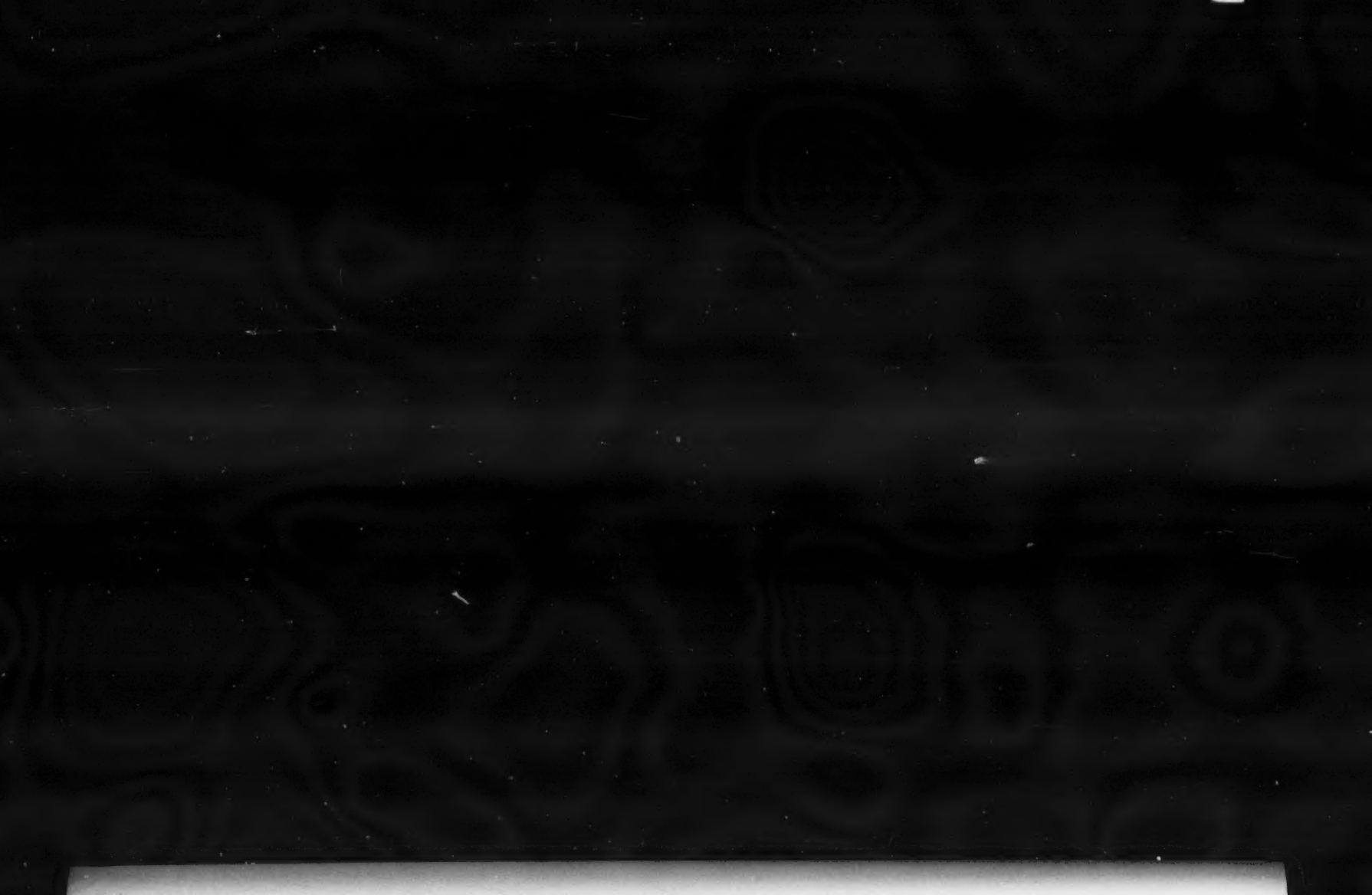
FIG. 8. Cylinder head (Fig. 6) with Fig. 7 screwed onto the column.

FIG. 9. Micro dish containing assay tissue culture which has grown for several days *in vitro*.

FIG. 10. Double membrane assembly ready for *in vivo* implantation; implantation period limited to 4 days. Assembly consists of Fig. 9 enclosed by Fig. 5 and two Fig. 4s.

FIG. 11. Lateral view of assembly in Fig. 10.

FIG. 12. Single membrane assembly ready for *in vivo* implantation; implantation period theoretically unlimited. Assembly consists of Fig. 9 enclosed by Figs. 5, 4 and 8.



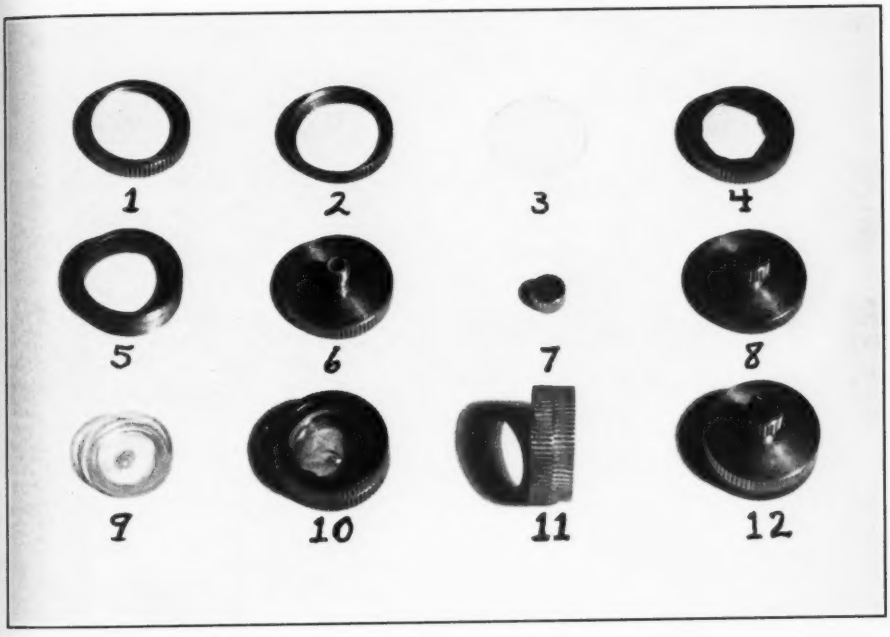


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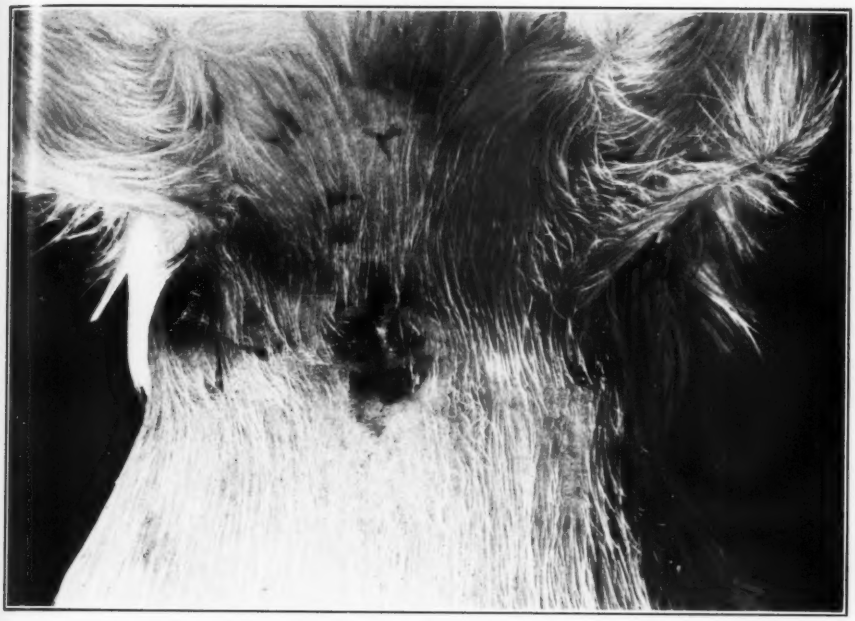
PLATE 112

FIG. 13. Photograph which illustrates the usual appearance after the cylinder implants have been in the subcutaneous tissues for several days. Assembly of Fig. 10 is in the left axillary region beneath the superficial cutaneous abrasion and assembly of Fig. 12 is in the right axilla. The sutures have been removed from the healing midsternal wound. The metal column of assembly of Fig. 12 projects through the skin and serves as an avenue through which plasma is added to the culture within the receptacle.

FIG. 14. This photograph shows the method of aspirating fluid from the implantation receptacle and of dropping fresh plasma onto the tissue culture in the micro dish which is properly oriented within the receptacle for this manipulation. This procedure is required about every 4 days during the period of implantation to keep pace with continuous lysis of the fibrin clot in which the assay culture is embedded.



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DERMATOMYOSITIS *

A Study of Five Cases

Thomas D. Kinney, M.D., and Mary M. Maher, M.D.

(From the Laboratory of Pathology, Yale University School of Medicine, New Haven, Conn.)

Dermatomyositis has been known since 1887, and while it is still comparatively infrequently observed it is of ever increasing interest. There are comparatively few autopsy studies reported. In this article 5 cases of dermatomyositis are presented, 2 of which have a complete postmortem examination. The literature is also reviewed.

Unverricht was the first to recognize the association of skin and muscle lesions and in 1891 gave the disease its present name of dermatomyositis. However, Wagner in 1863 described what was apparently a case under the heading of "a rare muscular disease." Polain published a case report in 1875 as "an atypical case of chronic glanders." In 1887, the year in which Unverricht first described this condition, Wagner reported a 2nd case as "acute polymyositis." At this time Wagner expressed the opinion that his 1st case was the same as acute polymyositis, but there is some doubt as to the validity of this interpretation since trichinae were detected in the muscle. Hepp (1887) reported a case which he termed pseudotrichiniasis, and Dr. Henry Jackson of Boston described Hepp's case in this country the same year. The 1st American case to be published was that of Jacoby in 1888. Senator described changes in the peripheral nerves in cases of polymyositis with skin involvement and named the disease neuromyositis. Oppenheim (1903) pointed out that the mucous membranes might also be involved and coined the name dermato-mucoso-myositis. Lorenz described a group of cases in which there was a hemorrhagic infiltration between the muscle bundles and noted also involvement of the heart muscle. Cases of this type were described as polymyositis hemorrhagica. In 1903 Steiner collected 27 cases from the literature and added 1 of his

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own. At this time he defined the disease as "an acute, subacute or chronic disease of unknown origin, characterized by a gradual onset with vague and indefinite prodromia, followed by edema, dermatitis and a multiple muscle inflammation." Batten described the 1st case in a child in 1912.

Almost every writer on the subject of dermatomyositis suggests a different etiological agent. Protozoa, a filterable virus, staphylococci, streptococci, the colon bacillus and the tubercle bacillus have all been mentioned. Some authors have suggested various toxins, allergic manifestations, endocrine disturbances and vitamin deficiencies. Still others have implicated the reticuloendothelial system. It becomes apparent after reviewing the literature that the pathogenesis of this disease is unknown.

Five cases of dermatomyositis are presented. In Cases 1 and 3 the postmortem examination is given, while Cases 2 and 4 are proved by biopsy. In Case 5 no biopsy could be performed but as it is such a typical case it is included here.

Case Reports

Case 1. The patient, P. A. (Hospital No. A73051), a 7 year old white boy, was first seen in the Pediatric Clinic of the New Haven Hospital in February, 1937, at the age of 5 years because of gonorrheal urethritis. The treatment consisted of large doses of theelin over a period of 4 months, together with circumcision. There was apparently a complete cure.

At this time the skin of the elbows, knees, and knuckles of the hands were red, rough and scaly. The skin of the face was covered by greasy macular scales. The child's family stated that these skin changes began at the age of 1 year. They were usually preceded by a blister, always over a bony prominence, which would be followed by the erythematous scaly lesion.

The child did not walk until the age of 16 months and was always somewhat unsteady on his feet, falling readily. He improved somewhat during his 3rd and 4th years, but when first seen in the clinic in his 5th year he walked unsteadily and with a wide base. The significance of the child's cries on palpation of the arms and legs could not be determined because of his irritability.

This condition remained unchanged until the late summer of 1937 when he suffered from a cold. Following this he began to have increasing difficulty in walking and complained of pain in the hips. During the next 7 months he gradually developed flexion deformities of the knees, inability to use his hands, and swelling of the arms from the fingers to the elbows. He suffered from malaise and anorexia, and showed marked irritability when handled. He also began to experience difficulty in swallowing. The child's diet was never satisfactory. There was a marked excess of carbohydrates with but little protein and vitamin-containing foods.

On admission to the hospital in April, 1938, the temperature was 101.2° F.,

pulse 156 and the respirations 26. The blood pressure was 158/128. For 2 days prior to admission he had had coryza, with drowsiness and fever. The child was poorly developed and nourished. There was edema with a patchy erythema of the skin of the face, and especially of the eyelids. The gums were raw with ulcerated areas about each tooth. There was a patchy desquamation of the tongue. The pharynx was red and filled with mucus. The skin of the palms and phalanges was pink, cold and clammy. There were contractures of the elbows and knees, and marked atrophy and weakness of the muscles of the legs and arms. The right masseter muscle also appeared to be atrophic. The muscles were painful to palpation and on motion. The arms could not be raised more than 90°. The abdominal reflexes were present but the cremasterics were absent. No deep reflexes could be obtained. The plantar responses were normal. There was nothing of note in the remainder of the physical examination.

Because of the poor nutritional state the child was given a high caloric, high vitamin diet. Due to the difficulty in swallowing, which grew steadily worse after admission, he was given gavages of concentrated food.

An attempt was made to correct the bilateral contractures of the knees by the application of plaster casts but he could not tolerate the pain and these were removed.

From the end of May until the first of August, 1938, was a period of gradual improvement. Motion gradually became bearable, the muscles became less tender, the temperature approached normal, and the patient ate well without the tube. Frequent blood transfusions were given. In July the plaster casts were reapplied with almost complete correction of the knee contractures. Seven doses of 40 mg. each of guanidine hydrochloride were given without effect.

Ten days before death there was a sudden exacerbation of the disease. The temperature suddenly rose to 105° F., and there were edema and erythema of the skin of the face. White atrophic patches appeared in the skin on the remainder of the body. Petechiae were noticed, first over the knuckles and the mucous membranes of the mouth, and finally over the entire body. The nasopharynx filled with mucopurulent material, and respiration and swallowing became extremely difficult. The patient became progressively more drowsy and weaker, and in spite of frequent infusions and transfusions died Aug. 10, 1938.

During his entire hospital stay the red blood count never dropped below 4,300,000 nor the hemoglobin below 70 per cent. His white blood count ranged from 7500 on admission to the neighborhood of 20,000 during the period of difficulty in swallowing and during the terminal stage. In the remission of May to August it dropped to 10,000. Eosinophils were noted in the smear several times, never rising above 4 per cent. Their presence did not coincide with acute episodes. The stools contained Ascaris ova both before and after hexylresorcinol therapy. The serum albumin and globulin values were as follows: on April 21, 1938, albumin 3.3 mg. per cent, globulin 3.9 mg. per cent; on May 24, 1938, albumin 2.7 mg., globulin 2.6 mg.; on July 12, 1938, albumin 3.3 mg., globulin 3.9 mg.; and on July 23, 1938, albumin 3.8 mg., globulin 3.4 mg. The blood sugar was 85 mg. and the cholesterol 190 mg. The values for calcium and phosphorus were 9.8 mg. and 3.7 mg. The urine revealed nothing of significance except the following:

On May 27 a 24 hour urine specimen with a volume of 700 cc. was examined and was found to contain creatinine 96 mg. and creatine 203 mg., giving a creatinine coefficient of 7.8. The blood cultures were consistently negative. The skin reaction to 1:50 and 1:10 mg. tuberculin was negative.

Postmortem Examination *

The body was emaciated. There were several areas of alopecia and in these the scalp was dry, thickened and scaly. There was marked edema of the skin of the face, neck, anterior thorax and upper extremities. Over the face the skin was scaly and thickened, with irregular fissures and excoriations and in a few places frank ulceration. The skin over the bony prominences of the extremities was thickened and erythematous. The proximal interphalangeal joints of the fingers and the distal interphalangeal joints of the thumbs were covered by red, thick fissured skin. There were decubitus ulcers over the bony prominences of the back and right thigh. The lower extremities were free from edema. The skin was dry and scaly and adherent to the subcutaneous tissues. There was tremendous atrophy of the muscles and the joints were correspondingly prominent. The gingivae were red and ulcerated. Otherwise the mucosa of the mouth was intact.

The muscles of the trunk were markedly decreased in amount and pale tan in color.

There was clear straw-colored fluid in both the peritoneum and the left pleural cavity. Small hemorrhages were present beneath the visceral peritoneum of the intestines and extending through the intestinal walls. The mucosa of the duodenum and of the jejunum was diffusely reddened. Five ascarides were found in this portion of the intestinal tract. Subpleural petechiae were present. No gross lesions of the lungs were apparent.

The heart weighed 95 gm. and did not appear unusual in any manner. The remaining viscera revealed no gross abnormalities.

Microscopic Examination

The lungs had small patches of atelectasis and emphysema. There was a small amount of periportal lymphocytic infiltration in the liver. The intestinal wall in some portions was infiltrated by a few polymorphonuclear leukocytes and the layers were slightly separated by edema. The blood vessels of the muscularis

* Autopsy 4341.

and subserosa displayed a perivascular collaring by an exudate which consisted of small lymphocytes and polymorphonuclear leukocytes. A few of the lumens of these vessels were occluded by thrombi. This change was most marked in the duodenum and upper jejunum where the ascarides were found.

Sections of muscle were taken from the right pectoralis major, the right and left pectoralis minor, the left iliopsoas, and the right rectus and the diaphragm. Both Zenker and formalin solutions were used as fixatives. Sections stained with hematoxylin and eosin, iron hematoxylin, phosphotungstic acid hematoxylin, sudan III, and by Masson's trichrome, Best's carmine and Gram's methods were studied. All the muscles showed lesions of a similar nature but of different degrees.

The extent of muscle involvement was most remarkable, almost all the muscle fibers being altered in some manner (Figs. 1 and 2). In many places the muscle fibers had an amorphous appearance with loss of the usual fibrillar structure. In these areas striations were lost, the fibers showed large and small vacuoles, or there was a loss of the sarcolemma nuclei. These changes occurred singly or in combination. In a few areas proliferation of the sarcolemma cells had taken place, while in other sites the sarcolemmal sheaths were the only remnants of the muscle fibers. The Masson stains clearly showed patchy increase of the endomysium, perimysium and epimysium.

There was widespread edema of all the tissue and especially of the connective tissue. This edema was marked, not only between the muscle bundles but between the individual muscle fibers as well. There were patches of exudate throughout the muscle bundles. The exudate was made up of small lymphocytes, plasma cells, an occasional monocyte and a rare polymorphonuclear leukocyte. There were similar but larger patches of exudate within part of the perimysium and epimysium. Some of the muscle fibers had been replaced by pale staining, fibrous connective tissue; a portion of this was very dense and closely knit, while another was rather loose and contained numerous fibroblasts. There was a marked perivascular infiltration by an exudate similar in character to that seen in the muscle. In some places the vessel walls were infiltrated by lymphocytes. The walls of many of the blood vessels were thickened with definite narrowing of their lumens. This thicken-

ing appeared to be due to the deposition of hyalinized tissue in the media (Fig. 3). The walls of the lymphatics were also involved by exudate similar to that seen in the muscles. A few of the lumens of the lymphatics contained fibrin and small lymphocytes together with an occasional polymorphonuclear leukocyte.

A section of the left ventricle showed well stained muscle fibers which were separated by edema and in which the cross striations were well marked. Imperceptibly merging into this relatively uninvolved myocardium was an area in which the edema was more marked and in which lymphocytes had infiltrated between the fibers and around the blood vessels. There the myocardium was still well stained and the cross striations were well marked. Throughout this area were patches where the muscle fibers were poorly stained and the cross striations had disappeared. Many of these fibers were shrunken or had been completely replaced by fibrous tissue. There were large numbers of small lymphocytes together with a few monocytes between the muscle fibers and surrounding the blood vessels.

The epicardium showed definite thickening which was due to fibrosis. There was a patchy infiltration of the subepicardial fat by small lymphocytes together with a few polymorphonuclear cells. There was no apparent involvement of the epicardium.

The myocardium of the right ventricle resembled that of the left ventricle in that it contained areas which ranged from apparently normal tissue to those with extensive involvement. Some muscle fibers took a very deep stain and were fragmented and diffusely dispersed. Here there were large numbers of small lymphocytes with a few monocytes and an occasional polymorphonuclear cell together with fibroblasts, proliferating capillaries and muscular débris. The walls of the arterioles and smaller arteries were thickened by the deposition of hyalinized tissue in their media. There was consequent narrowing of the lumens of these vessels.

Skin from the right knee and elbow and the left wrist was examined. That from the right elbow showed varying changes. In some places there was almost complete atrophy of the epidermis (Fig. 4). A few layers of keratinized material admixed with red blood cells were present on the surface, representing the stratum corneum. The long axis of the nuclei of the epidermal

cells was parallel to the surface. No evidence of granular or pigment cells was discernible. The dermal papillae were flattened and broad. In some places they penetrated to within one epithelial cell layer of the surface. In other parts of this same section a somewhat thicker layer of keratinized material, the stratum corneum, was present. The stratum granulosum cells were sparse and irregular in distribution. The stratum malpighii had its usual structure but many of the cells were vacuolated, especially those in the basal layer. These were the so-called "balloon cells." Here, too, the orderly arrangement of the epidermal cells was not preserved and many of the cells showed evidence of degeneration. Mitotic figures were present in the stratum malpighii. The dermal papillae were flattened. The derma showed evidence of increased compactness. The usually loosely woven connective tissue and elastic fibers were bound together by fibrous connective tissue, much of which had a hyalinized appearance. The fibers in the papillary portion and the outermost part of the reticular portion presented a rose colored, almost amorphous appearance. Continuous with the derma and extending into the underlying fat were broad sheets of tissue which in some places had a densely collagenous character. The latter tissue was infiltrated with scattered polymorphonuclear leukocytes. Groups of red cells in various stages of change were found almost everywhere in this tissue. Occasional monocytic cells were found about the blood vessels and in the fibrous tissue. There was an extravasation of red blood cells among the fat cells of the panniculus. In some places the fat cells were separated by homogeneous pink staining material which appeared to be continuous with the septums previously mentioned. Hair follicles and glands were bound into the connective tissue.

In a hematoxylin and eosin stain of skin from the right knee the stratum corneum was moderately thick. Between the stratum lucidum and stratum granulosum were vesicles. The stratum granulosum was present and was one layer in thickness. Within the stratum malpighii were "balloon cells," many in the basal layer, others farther out toward the lumen. The stratum malpighii was thinned, being only three to four cells in depth. Mitotic figures were present in this layer. The dermal papillae had a broad, spade-like appearance. The derma was more closely woven

than usual, which was due to an increased amount of fibrous connective tissue. The arrectores pilorum were normal. A slight infiltration of small round cells, polymorphonuclear leukocytes and fibroblasts was present, particularly in the perivascular and periglandular connective tissue. The subcutaneous adipose tissue was also infiltrated. Fibroblastic proliferation was proceeding here. The capillaries were distended with red blood cells. In some of the small vessels of the upper derma the adventitia had a peculiar, granular, pink staining character and the walls of these vessels were infiltrated by lymphocytic cells. In other places there was perivascular collaring with some evidence of hyalinization. There was a layer of necrotic tissue between the panniculus adiposus and the derma. One vessel seen at the junction of the panniculus and the derma lay within this necrotic tissue which had been infiltrated with polymorphonuclear leukocytes. The lumen of this vessel was completely obliterated by a mass of bright pink staining, amorphous material. The structure of this vessel was similar to that seen in malignant nephrosclerosis (Fig. 5).

The skin from the left wrist resembled that of the right elbow. However, there were no vesicles. There was somewhat more edema and less fibrous tissue proliferation of the derma and a less marked cellular exudate in the subcutaneous tissues. The sweat glands had thickened basement membranes and were embedded within dense connective tissue containing scattered mononuclear elements. Deep in the derma the lymphatics were widely distended.

There were rather large collections of lymphocytes and plasma cells in the perineurium and perineural connective tissue of the femoral nerves. Neither these nerves nor any of the other peripheral nerves were infiltrated with cells among the myelin fibers. The whole inflammatory process was, therefore, a perineural one. Marchi preparations of the sciatic, femoral and brachial nerves disclosed a slight degree of fatty change. In Spielmeyer and Kulschitzky preparations the demyelination was of a moderately extensive degree. The absence of fat in sections stained with sudan III, together with the findings in the Spielmeyer and Kulschitzky preparations, indicated that the myelin destruction was an old chronic process. The spinal cord was intact, both as regards the cellular and the myelin components.

Sections of muscle were stained by the Larvelle-Renmont technic to demonstrate the nerve endings. Even where there was extensive degeneration and atrophy, the nerve endings were well preserved.

Aerobic and anaerobic postmortem cultures of the spleen, liver, right kidney, pectoral muscle, and skin and muscle from the right knee and elbow, were negative.

Comment

Every attempt to discover some possible etiological factor in this case was unsuccessful. The tuberculin test was negative and there was no clinical or postmortem evidence of tuberculosis. All cultures of the muscles, blood and viscera were sterile. The stools were persistently positive for ascaris in spite of vermifuges, but this could hardly be of significance in the clinical course of the disease. It is interesting to note that the child apparently suffered from this disease from the age of 1 year, with remissions and exacerbations until the time of his death at the age of 7. For this reason it is extremely doubtful that the gonorrheal infection was an etiological factor.

All therapeutic efforts were disappointing. Guanidine hydrochloride was used because of its value in the muscular dystropies but there was never any evidence that it decreased the muscular weakness.

Case 2. Mrs. E. R. (Hospital No. A78950), a 26 year old, white married woman, was referred to the New Haven Hospital on May 26, 1938, because of loss of strength in her legs. The family and past histories were noncontributory.

Seven months prior to admission the patient became listless and lost her desire for food. Within the next 2 months she lost 20 pounds in weight. Five months before admission she began to vomit occasionally. Shortly after this she started to complain of weakness and fatigue of the legs when walking. She also noticed that the skin over the thighs was becoming increasingly hard. These symptoms became steadily worse and she was admitted to the local hospital where a diagnosis of multiple sclerosis was made. She was given a series of eighteen intramuscular injections of Betaxin (synthetic crystalline vitamin B_1 hydrochloride) but continued to grow worse, the muscles of the legs becoming so tender that pressure of the bed clothes was painful.

The physical examination on admission revealed a temperature of 98.6° F., pulse 110 and respirations 22. The blood pressure was 130/80. As she was lying in bed she very carefully avoided motion of the lower extremities. There was some injection of the cutaneous minute vessels over the face, a few papules were present in the folds of the neck and a papular eruption was

found all over the upper back. The skin over the legs had a strikingly glossy appearance with some atrophy. The skin of the feet was atrophic and a violaceous color with a blotchy distribution. Here the skin was rather cold but elsewhere it was warm and dry. The skin over the forehead and lower extremities was adherent to the subcutaneous tissue. The mucous membranes were uninvolved. There was no edema or other abnormalities of the arms. A bilateral foot drop was present with brawny edema of the legs and thighs but not of the feet. The calf and thigh muscles were doughy and edematous in consistence. Any motion of the knee joints was exquisitely painful. Motion of the patella was not painful. Both the dorsal and plantar flexors of the ankles were weak although some motion was possible in both. Motion of the ankles without motion of the knees was not painful. Plantar, but not dorsal flexion of the toes was possible. The muscles of the thigh and calf could not be tested for strength because of pain. The arm muscles as far as could be determined clinically were intact. Position and vibratory senses were uninvolved and no cutaneous hyperesthesia was noted. The reflexes of the leg could not be tested because of pain; the others were in order. The eyes showed some nystagmus on lateral deviation. The gag reflex was present and the uvula ascended in the midline on phonation. The heart and lungs presented nothing unusual. The liver edge was two fingerbreadths below the costal margin but the spleen was not palpated.

Roentgenographic examination of both knees and both ankle joints revealed a marked degree of osteoporosis of an acute type.

The patient's course in the hospital was one of slow but steady improvement. She had plaster casts applied to the feet and legs with some improvement of the foot drop. She received large doses of Betaxin and physiotherapy treatments consisting of active and passive assisted exercises to the legs and massage. The muscle strength increased and the pain almost completely subsided in the extremities. Her course was afebrile throughout. She remained in the New Haven Hospital for 87 days.

Seven months after discharge she was again seen at the New Haven Hospital for examination. In the interim she had been receiving massage and active assisted exercise. There had been a remarkable increase in activity and freedom from pain. She was able to walk 4 months after leaving the hospital. The only complaint was that of tiring easily. There was slight atrophy of the thigh but no edema. No tenderness of the muscles or sensory changes were noted. The skin over the calves was thin and shiny; the muscles of the calf were not tender although somewhat atrophic. Bilateral foot drop was present with brawny edema of the dorsum of the feet and ankles, and extending up the lower quarter of the tibia. Muscle strength and flexion of thighs on the abdomen, flexion and extension of the knees, abduction and adduction of the thighs were good. Dorsiflexion of the feet was weak but the plantar flexion was good. The knee and ankle jerks were absent but the other reflexes were in good order. The patient showed a typical foot drop gait.

Laboratory studies during her hospital stay revealed a red blood count of 4,000,000 and a hemoglobin of 90 per cent. The urine and stools were negative except for an occasional red blood cell in the former. The white blood count averaged about 10,000 with 75 per cent polymorphonuclear leukocytes and 1 to 5 per cent eosinophils. The albumin of the serum was 2.95 mg. per cent and the globulin 2.73 mg. per cent. The non-protein nitrogen was 30 mg. and

the calcium and phosphorus 10.41 mg. and 4.26 mg., respectively. On her return visit to the hospital her white count was 7000 and the eosinophils 3 per cent.

Biopsy

A biopsy was taken from the lower medial portion of the muscle of the right calf under cyclopropane anesthesia on June 3, 1938. Grossly the skin did not appear unusual in any respect. There appeared to be moderate edema of the subcutaneous fat and connective tissue. The portions of the soleus and gastrocnemius muscles taken were markedly pale and gelatinous in appearance.

Microscopically the skin showed a moderate diminution of the thickness of the stratum corneum. The cells of the stratum granulosum were sparse and uneven in distribution. The cells of the stratum malpighii were definitely diminished in number, being only two to four cells in depth. The usual orderly arrangement of these cells had been lost and the basal layer was not well demarcated. Throughout the epidermis, and especially in the basal layer, there were many vacuolated or so-called "balloon cells" whose cytoplasm had almost completely disappeared, but the cellular outline was still very prominent. The nuclei of these cells took a deeper basophilic stain than the surrounding cells. Only an occasional pigment cell was found in the epithelium. There was definite flattening of the dermal papillae and they were much wider than usual. The dermis was very edematous and an occasional small round cell was found. A rare polymorphonuclear cell and a few small round cells were arranged about the sweat glands and in the perivascular spaces.

Sections of both muscles showed the muscle fibers to be partially degenerated and widely separated from each other by an increase in loosely arranged endomysium. The perimysium was likewise increased. There was a diffuse lymphocytic infiltration and in some areas there were dense collections of lymphocytes. There was proliferation of sarcolemma cells. Vacuolization of the muscle fibers was prominent.

Sections of muscle were stained by the Larvelle-Renmont technic to demonstrate the nerve endings. These were well preserved, even in places where there was extensive degeneration and atrophy.

Comment

This patient is apparently in a remission at the present writing. However, she is still incapacitated by weakness and by bilateral foot drop, as well as by brawny induration of the lower extremities. Her course was afebrile, which is rather unusual in a case showing such marked involvement. The degree of osteoporosis demonstrated by X-ray was quite remarkable. Because of the pain on motion of the knee and ankle joints this case could possibly be confused with arthritis had it not been for the X-ray findings. With atrophy and wasting in the muscles the joints appear more prominent and this occasionally causes the disease to be confused with arthritis, but whenever a true arthritis is present, it would appear that it is merely coincidental.

CASE 3. The patient, a 60 year old housewife (Hospital No. A91379), entered the hospital on Dec. 30, 1938, complaining of cough, weakness and loss of weight. The family and past histories contained nothing of interest except that the patient developed a "skin cancer" on her nose 1 year before admission. This lesion disappeared after X-ray treatment.

The present illness dates back to 7 years before admission when the patient suffered from multiple carbuncles. She estimated there were 200 of these in the course of 18 months. This was followed by a generalized eczema which was most marked on the back, abdomen and hands. At this time she was found to have an elevated blood sugar. However, sugar was never found in the urine until 8 months prior to admission.

Five years prior to admission the patient developed an "arthritis," *i.e.* swelling and limitation of motion of the knees, shoulders and hands. The joints were never red but the patient feels there was some deformity present.

For 2 years before admission the patient's leg and back became progressively weaker and for 1 year she was unable to raise her head without supporting it with her hands. For the 6 months before admission she coughed after eating, choked frequently and had difficulty in swallowing. In addition, her voice changed.

On admission the temperature was 98.6° F., respirations 26 and the pulse 86. The blood pressure was 170/90. The patient was thin and emaciated and appeared chronically ill. The skin showed scaly erythematous lesions over the hands, arms, back, abdomen and legs. It was loose and inelastic. There was a bilateral ptosis and internal strabismus. The edges of the tongue were smooth and the muscles of the jaws were weak. There was a definite nasal character to the voice. She was unable to support the neck alone. There was no demonstrable excursion of the diaphragm. There were sibilant and sonorous râles throughout both lungs with fine râles at the left base. The heart was not enlarged and the sounds were of good quality. There was a fairly rough systolic murmur at the base. The liver was felt two fingerbreadths beneath the costal margin and the spleen was palpable. The joints of the fingers and knees were slightly enlarged and there was slight limitation of motion. There

was generalized atrophy and weakness of the muscles but no tenderness. The reflexes were equal but hypoactive. There were no sensory disturbances. The axillary, cervical and inguinal glands were palpable.

Two days following admission to the hospital the patient developed signs of bronchopneumonia with a temperature of 104° F. This was confirmed by X-ray. The sputum was found to contain type IV pneumococcus, and 200,000 units of type specific serum were administered. In addition the patient was given sulfapyridine. Her temperature dropped to around 100° F. but she became progressively weaker and died Jan. 8, 1939.

It is interesting to note that although she had an elevated blood sugar while in the hospital there was no glycosuria. The blood Kahn was negative.

On admission the hemoglobin was 80 per cent, the red blood cell count was 4,640,000, the white blood cell count 3350, with 61 per cent polymorphonuclear leukocytes, 34 per cent lymphocytes, 4 per cent eosinophils and 1 per cent basophils. The blood cell morphology was not remarkable. After the bronchopneumonia developed, the white blood cell count was 20,160, with 91 per cent polymorphonuclear leukocytes, 5 per cent lymphocytes, 2 per cent monocytes and 2 per cent eosinophils. The white count gradually dropped to 9500 on the day of death.

Repeated urine examinations were negative except for a 1 plus albumin and an occasional white blood cell. The blood sugar was 151 mg., the nonprotein nitrogen 34 mg.

Postmortem Examination

The body was that of a well developed, poorly nourished, elderly white woman. The skin around the mouth and the upper lip was marred by many crusted, dark brownish gray, scab-like areas which were confluent in many places. The skin over the arms was wrinkled, shiny and parchment-like. The skin over the hands was rather tight and when the hands were flexed the skin had a blue tinge. The skin over the trunk was dry, rough and scaly. Aside from the lesions described, the skin was everywhere intact and grayish white in color. The hair distribution was that of a normal female. There was a bilateral hydrothorax. Both lower lobes of the lungs were shrunken and doughy, and there were focal areas of consolidation throughout. The heart weighed 388 gm. and was of the usual size and shape. There was no evidence of a valvular lesion or of hypertrophy or dilatation. The spleen weighed 460 gm. and measured 16 by 10.5 by 4 cm. The organ was quite firm. There was an infarct at the lower pole of the right kidney. Nothing of note was found in the remainder of the organs.

Aerobic and anaerobic cultures of the heart's blood, spleen and liver were sterile.

Microscopic Examination

The lungs showed areas of focal pneumonia. There were also small infarcted areas with organizing thrombi.

A section of the left ventricle showed well stained muscle fibers in which the cross striations were well marked. In a few focal areas there were edema and moderate swelling of the muscle fibers. These areas were infiltrated by lymphocytes and there was minimal degeneration of the muscle fibers, accompanied by an increase of the fibrous connective tissue in the endomysium and perimysium (Fig. 6).

Tissue was taken from the tongue, diaphragm, rectus, gluteal, splenius capitis and iliopsoas muscles. Both Zenker and formalin solutions were used as fixatives. These sections were stained with hematoxylin and eosin, Masson's trichrome stain, iron hematoxylin, phosphotungstic acid hematoxylin and the Gram stain. The sections showed tremendous involvement of the muscle fibers. The majority were swollen, even those in which the cross striations were well preserved. In the hematoxylin and eosin sections the color varied from a deep rose to a pale pink. Many of the muscle fibers were amorphous in appearance, and it was in these fibers that the cross striations were lost. This was well shown by the iron hematoxylin stain. There was a slight degree of vacuolization in a few of the muscle fibers, and in some there was breaking up and fragmentation. In some places the sarcolemma was faded and broken; in others it was the only remnant of the muscle fiber. There was proliferation of the sarcolemma cells in a few areas. The Masson stains showed patchy but moderate increase in the endomysium, perimysium and epimysium. Some of the muscle tissue had been replaced by scar tissue. An exudate of small lymphocytes together with a few monocytes and plasma cells had diffusely infiltrated the muscle fibers in many places (Fig. 7). There were large foci of these cells in the endomysium and perimysium, together with large accumulations in the perivascular and perilymphatic spaces. Some of the vessel walls were infiltrated by small lymphocytes. A few of the vessels showed proliferation of the media with thickening of the walls.

The skin from the scapular region showed two types of change. In the first the epidermis had undergone almost complete atrophy.

There were but a few layers of keratinized tissue representing the stratum corneum. The nuclei of the epidermal cells were arranged so their long axis was parallel to the surface. The epidermal cells were discoid in appearance. There were no granular or pigment cells present. The dermal papillae were broad and flattened. These papillae were separated in places from the surface by but a single layer of epithelial cells.

In the second type of change the keratinized tissue of the stratum corneum was thicker and the stratum granulosum cells were sparse and irregular in distribution. There was vacuolization of many of the cells, particularly those of the basal layer. These were the so-called "balloon cells." There were moderately large numbers of mitotic figures present in this layer of the epidermis. The dermal papillae were broad and flattened. In both types of change described in the epidermis the derma appeared denser because the usual loosely woven connective tissue and elastic fibers were firmly pulled together by fibrous connective tissue. Much of the connective tissue had become hyalinized. There was an occasional cell of the monocytic variety in this fibrous tissue and around the blood vessels of the derma. The hair follicles and glands of the derma were caught up in this dense fibrous tissue. Some of the fat cells of the panniculus were separated by homogeneous pink staining material. There was marked perivascular collaring of the small vessels situated deep in the panniculus by small lymphocytes.

The skin from the pubic region showed essentially the same changes seen in the skin from the scapular region. There was tremendous flattening of the papillae and thinning of the epidermis (Fig. 8). The fibrous tissue of the dermal papillae had a much denser homogeneous character than is seen in normal skin and in many areas it penetrated almost to the skin surface. The typical lymphocytic infiltration of the fibrous tissue and about the blood vessels of the skin and panniculus was quite marked.

All the peripheral nerves disclosed a moderate degree of demyelination which was demonstrable in both the Marchi and the Weigert preparations. There was, however, no evidence of an inflammatory process in these nerves.

The spinal cord, medulla and pons were normal as regards the cellular components and the nerve fibers.

Several of the cranial nerve nuclei in the floor of the fourth ventricle contained perivascular cuffs of lymphocytes as well as interstitial lymphocytic infiltration. The neurons were injured and were the sites of glial proliferation resulting in small nodules (Fig. 9). These lesions occupy a symmetrical position bilaterally. A similar perivascular change was found in the cortex of the frontal lobes. There were no changes of this type or other types elsewhere in the nervous system.

Comment

This patient was in the hospital for such a short time and was so ill that complete studies could not be carried out. The cause of death was bronchopneumonia following involvement of the muscles of deglutition which is the most common cause of death in dermatomyositis. In view of the postmortem findings in the central and peripheral nervous systems the absence of clinical neurological symptoms is interesting. The absence of muscle tenderness in the presence of such marked involvement is of interest.

The disturbed carbohydrate metabolism deserves emphasis. This disturbance was of long standing as it was discovered early in the course of illness, 6 years before death. There was a leukopenia at the time of admission. However, once the bronchopneumonia developed there was a definite leukocytosis.

Sulfapyridine was used in the treatment of the pneumonia. There was no evidence that it influenced the skin or muscle lesions although, of course, it is unfair to attempt to evaluate results after such a short trial.

CASE 4.* Mrs. M., a 65 year old housewife, had been in excellent health until Jan. 6, 1939. At this time she complained of sore throat, with pain about the muscles of the right shoulder and neck. The physical examination was negative except for a moderately inflamed throat.

Four days later, on January 10, the patient complained of increasing pain in the neck and shoulder, and also of pain in the muscles of the calf. The temperature at this time was 100° F. The white blood count was 13,300. The urine was negative. Beginning Jan. 13, 1939, 7 days after the onset of the disease, the patient had 38 days of severe illness with fever reaching 103° F., and showing daily fluctuations. During this time there was puffiness of the face, a subconjunctival hemorrhage, and she suffered from diffuse tenderness and stiffness of the muscles. During the latter part of this period the skin became rather firm and slightly edematous. There was no evidence of joint

* We wish to thank Drs. Paul Rosahan and John White of New Britain, Conn., for the opportunity of including this case in our series.

involvement. There was considerable distention but no diarrhea or other gastro-intestinal tract symptoms.

The question of trichinosis was naturally raised. The patient remembered eating rare roast pork approximately 2 weeks prior to the onset of her illness. However, her husband and children also ate the same meat without ill effect. On Feb. 12, 1939, a biopsy of the left soleus muscle was performed under local anesthesia. Forty-three sections were made and examined without any evidence of trichina. The description of this specimen is given below. A skin test with potent trichina antigen was negative. This was performed in the 4th week of the illness. However, the biopsy findings did confirm the clinical suspicion of dermatomyositis.

The patient was given neoprontosil, 60 grains a day for 2 days, and 30 grains a day for 8 days, beginning Feb. 7, 1939. There was no evidence that this drug had any effect on the course of the disease and it was discontinued. She was given salicylates without much relief. The remainder of the treatment consisted of iron and reticulogen for the anemia and pantopon for the pain. Dry heat applied to the painful areas in the muscles appeared to be of some value.

As the disease progressed, small, soft, non-tender, non-fluctuant nodules were found in the subcutaneous tissue. These were especially marked in the antecubital fossae. Also, the patient complained of both generalized and localized sweating.

By the end of the 7th week of the illness the pain and rigidity of the muscles began to grow less marked. The course was then one of slow improvement with occasional local exacerbations of the pain and swelling of the muscles. The patient for a period of a week complained of photophobia and lachrymation of the right eye; 3 months after the onset of illness it was noted that the skin of the hands was tender and blue. By the 4th month the entire skin had assumed a dusky hue and there was frank cyanosis of the skin of the hands. In addition, the skin of the hands appeared quite thin and slightly scaly. By the 4th month atrophy of the muscles of the arms, legs, and especially of the interossei, had become quite marked.

During the middle of May the patient began to sit in the sun. Within a few days the exposed portions of the patient's arms and legs became deeply pigmented. The patient states this is very unusual as she never responded to sunlight in this manner at any time prior to her illness. By this time she felt much improved and was able to go about her housework; the only handicap was some limitation of motion of the fingers and shoulders and pain in the shoulder.

The following laboratory studies are of some interest. On Jan. 19, 1939, the agglutination tests for typhoid, paratyphoid and brucella were negative; on Jan. 28, 1939, the Kline test was negative; on April 10, 1939, the sedimentation rate was 68 mm. per hour. The white blood cell count ranged from 13,300 at the onset of illness to 56,300 at the height of the disease. The eosinophils varied from 2 to 6 per cent. The hemoglobin was 78 per cent and the red blood cell count 4,000,000 at the onset. It fell slightly as the disease progressed. The urine, except for a slight trace of albumin, was always negative. On June 5, 1939, the blood sugar was 90 mg., the non-protein nitrogen 63 mg., and the creatinine 2.5 mg.

Biopsy

A small biopsy was removed from the calf muscle and examined. The tissue was cut in several sections and every tenth section was mounted and stained. A total of forty-three sections was examined. Three of these revealed small focal zones of mononuclear cell infiltration in the loose connective tissue between the striated muscle fibers. Some of the muscle fibers displayed a decrease in density of the staining of their striations, but no true necrosis was seen. The arterioles were not involved in the inflammatory process. Unfortunately a piece of skin was not obtained.

Comment

This case appears to be in remission at the present writing. Perhaps the most interesting feature is the pigmentation of the skin following exposure to sunlight. It might be mentioned here that Turner describes pigmentation and skin lesions in 2 of his cases following exposure to sunlight.

It should be noted that blood creatinine is moderately increased. This is in keeping with the disturbances in the creatine metabolism discussed below.

In this case, as in the others, all types of specific therapy were disappointing. Although given in adequate amounts, sulfanilamide had no apparent effect on the course of the disease or on the skin and muscle lesions.

CASE 5.* The following case is presented briefly because of its typical onset and course. Unfortunately the patient would not permit a biopsy to be performed, but the nature of the skin and muscle lesions is such as to permit the diagnosis of dermatomyositis to be made with almost complete certainty.

Mrs. P., the patient, a 77 year old white woman, was well until July, 1938, when her legs became painful and swollen. At the same time the skin became thickened and scaly and the patient suffered from marked pruritus. In addition, erythematous areas occasionally appeared over the skin and the patient complained of a "burning" sensation in these areas. After several days these would disappear. There never has been any fever. The past and family histories were negative. There was no history of ingestion of pork or of previous infection of skin or muscle disease.

On physical examination there were several large erythematous areas scattered over the surface of the skin. The skin was thin and hard, had the appearance of crepe, and was not unlike that seen in senile atrophy. The

* This patient was never in the hospital and was seen at her home through the kindness of Dr. Maurice Strauss of New Haven, Conn.

patient walked and moved all extremities slowly and with effort. There was a lumpy infiltration in the muscle tissue and the examiner received the impression of lack of muscle tone and stated that the muscles felt "doughy." The remainder of the examination revealed nothing of note.

The course was one of slow progression. The patient has had recurring difficulty and pains in the muscles on movement until she has become bedridden. However, the swelling has disappeared, together with the occasional erythema of the skin. The pruritus has continued unabated. At the present writing, 10 months after the onset, there is marked muscular atrophy, the lumpy infiltration of the muscle is still present, and there are marked contractures at the knees, elbows and hands. The skin is thin and scaly and there is marked pruritus.

The following studies were made 10 months after the onset of the disease: non-protein nitrogen 34 mg., blood sugar 89 mg., total serum proteins 5.78 per cent, albumin 3.86 per cent, and globulin 1.92 per cent. A blood culture was sterile. The blood Kline test was negative.

Comment

Of note is the chronic course, the muscle swelling, infiltration and pain, with its resultant atrophy and contractures, and also the pruritus and atrophy of the skin. Edema was never a marked feature in this case and it is of interest to note the normal serum protein determination, which contrasts with the low determination found in the cases with edema.

Discussion

Considerable confusion as to its exact nature has existed since this disease was first described. For this reason several subdivisions have been made, *i.e.* the "acute hemorrhagic polymyositis" of Lorenz, the dermato-mucoso-myositis of Oppenheim, and the neuromyositis of Senator. It would seem that these are all part of the same disease entity. Cases from the literature as well as our own show evidence of myocardial involvement in true dermomyositis which is the main diagnostic criterion Lorenz uses to set aside his "acute hemorrhagic polymyositis" as a separate entity.

There are also instances of involvement of the mucous membranes in typical cases of dermatomyositis which would make the term "dermato-mucoso-myositis" superfluous. Our own studies and those collected from the literature point strongly to the fact that the peripheral nerve lesions are secondary to the muscle degeneration and atrophy. In Case 3, where there were inflammatory changes in the central nervous system, they were apparently of the same nature as the lesions in the muscle. For this

reason the term neuromyositis appears unjustified. It might be well to point out here that many of the older cases in the literature designated "neuromyositis" are most likely peripheral neuritis and dermatitis due to vitamin deficiencies.

There is considerable similarity between dermatomyositis and scleroderma. However, the muscle lesions in dermatomyositis appear to be of sufficient diagnostic import to warrant the separate classification of the two conditions for the present.

Bamber, Dowling, Ingram and Stewart, Witts, and Semon have described cases in which there were skin changes resembling poikiloderma atrophicans vasculare associated with the muscle changes commonly found in dermatomyositis. According to Witts and Bamber, such cases represent dermatomyositis and are distinguished from true poikiloderma atrophicans vasculare by the muscle lesions. Zoon, however, in a case of poikiloderma without any clinical manifestation of muscle lesions, found the type of change observed in dermatomyositis by biopsy of a muscle. McCarthy also points out that the skin changes in poikiloderma are often accompanied by localized edema, especially of the eyelids, and involvement of the muscles. We have been unable to find in the literature any other description of the muscle in a case of poikiloderma. It would seem that Zoon's work points out the necessity for further study of the muscle in cases of poikiloderma.

The vascular lesions have been the subject of considerable discussion. Lépine felt that in those cases in which there is thickening of the walls of the small arteries and lymphocytic infiltration with consequent narrowing of the lumens, the term angiomyositis should be applied. He considered this a distinct entity. This does not appear to be sufficient evidence on which to base a separate classification, since these vessel lesions form an important part of the pathological picture of dermatomyositis.

The disease has been reported from all parts of the world, the majority of cases from England, Germany and the United States. Any age may be affected but the majority are in the second to fourth decades of life. Patients have been stated to range in age from 2 days (Demel) to 77 years. The sexes are about equally involved.

The onset of the disease is usually insidious, but it may appear with dramatic suddenness. It often begins with malaise, fever,

anorexia, and vague aching pains in the extremities. This prodromal period is usually short but may be protracted. These initial minor symptoms are then followed by the two major complaints — skin lesions and muscular pain and weakness.

The skin changes may be of a wide variety but are usually of an erythematous type. No matter what type the lesion may be, there is almost always edema of the skin at some time in the course of the disease. The edema may be board-like or doughy in consistence and may or may not pit upon pressure. Edema of the face and eyelids is a frequent symptom. The skin may desquamate or become dry and scaly. Pruritus is not uncommon. Hyperesthesia may occur, as may dermatographism. Pigmentation of the skin may appear at any time during the disease.

The muscles become swollen and tender to touch and painful on motion. Any muscle or group of muscles may be involved. The most common sites are the muscles of the extremities and trunk. In the more severe cases, particularly in the terminal stages, the muscles of deglutition are frequently involved and rarely the accessory muscles of respiration, in which instance the patient may die a respiratory death. As the disease progresses the swelling usually subsides and indurated areas become palpable. This is followed by replacement of the muscle by scar tissue with resultant contractures. All these stages, *i.e.* swelling, induration and contracture, may exist simultaneously in the same patient, or indeed in the same muscle. As would be expected with contractures and consequent fixation of joints, the remaining muscle tends to become shrunken and atrophied from disuse. At this stage of the disease muscle weakness is a prominent feature. It is well to remember, however, the muscle weakness may be one of the first symptoms to appear, even before the skin manifestations.

Many writers describe similar changes in the myocardium, but clinical evidence of heart disease is uncommon. Cases have been described in which there are irregularities of the pulse. Occasionally an electrocardiogram may show evidence of myocardial damage.

There may be involvement of the mucous membranes. Stomatitis and angina are quite common. Clinically there is usually very little evidence of involvement of the nervous system. The tendon reflexes are altered only if there is disease in corresponding

muscles. Except in the severe and terminal stages of the disease, the sensorium remains clear.

Many writers have pointed out that there may be a quantitative decrease or even complete loss of excitability to electric stimulation. The mechanism of this is a matter of controversy, some declaring it is due to the resistance which edema offers to electric stimulation, while others state this is due to nerve lesions.

The spleen is often enlarged. It may decrease in size during a remission and increase during an exacerbation. There is usually fever, more commonly of the intermittent type. A persistently high fever is a bad prognostic sign. The pulse and respiratory rate follow the fever. General or localized hyperhidrosis is common.

There is no constant blood picture. The white count may be normal or there may be a leukocytosis or a leukopenia. The differential count is usually within normal limits. Occasionally there may be an eosinophilia, but this almost always occurs in chronic and rarely in acute cases. The urine is usually negative except for a trace of albumin. This slight albuminuria is no more than is seen in other diseases associated with fever. Rarely there may be red blood cells and casts in the urine.

The creatine metabolism has been studied. In the cases reported in the literature there was creatinuria, even with a decrease in the total creatine intake. Chemical examination of the muscles revealed a decrease in the creatine content. There may be a disturbance of the carbohydrate metabolism as several cases have been reported in which there was either a high blood sugar level, or a diabetic sugar curve was present.

Reports of serum protein dimunition in the literature are limited. McAlpine reported an albumin-globulin ratio of 3.39 over 2.79 in the early stage of the disease when the edema was most marked. Six months later, during a period of improvement, the ratio was 4.25 over 2.55. In 2 of our cases (Cases 1 and 3) the serum proteins were low and the albumin-globulin ratio was disturbed. In each case edema was present. In Case 5, without edema, the serum protein was normal.

The disease is most commonly confused with trichinosis, scleroderma, lupus erythematosus, erysipelas, and periarteritis nodosa. It may run an acute, a subacute, or a chronic course. Any of these

stages may end in death or progress to remission or recovery. The prognosis is always grave, as death occurs in approximately 50 per cent of the cases. The prognosis is worse in those cases in which there is involvement of the muscles of deglutition, the diaphragm and intercostals, or the myocardium. The morbidity among the survivors is very high, roughly 75 to 80 per cent. Atrophy, fibrosis and contractures of the affected muscles may make a chronic invalid of the patient. Any prognosis should be extremely guarded as relapses are frequent and recurrences may take place at any time, one of Turner's cases recurring after a remission of 8 years.

All therapeutic agents have been universally disappointing. Quinine, neosalvarsan, calcium chloride, arsenic, autogenous vaccines, electric stimulation, salicylates, urotropin, thyroid extract, iodides, parathyroid extract, intravenous typhoid vaccine, prostigmin, ephedrine and guanidine have been used with indifferent results.

The skin changes in dermatomyositis are not specific for this condition. The cardinal changes are atrophy of the epidermis, flattening of the dermal papillae, edema and fibrosis of the derma, and lymphocytic infiltration, usually most marked in the perivascular spaces and around the sweat glands and hair follicles. More rarely the vessel walls are thickened and show hyalinization or necrosis. Many writers have described vacuolization of the cells of the epidermis and state this condition is most marked in the basal layer. They have termed these vacuolated cells "balloon cells." These have been found in all of our cases in which the skin was studied, but it is most likely that they may be due to artefact as the same type of change is occasionally seen in normal skin.

Grossly the muscles are usually pale and soft. However, they may vary in color from gray through yellow to orange and they may be rubber-like in consistence. The muscle changes are of a non-specific, non-inflammatory nature. They consist of edema, focal lymphocytic infiltration and degeneration. Rarely, minute hemorrhages are present between the muscle bundles and fibers. In the chronic or healed stages the muscle bundles are partially or completely replaced by fibrous tissue. With all the above changes there is usually a perivascular infiltration by small round cells.

There may be changes in the heart muscle which are identical in every way with those seen in the skeletal muscle.

In some cases of dermatomyositis the peripheral nerves may show a moderate degree of demyelination which can be demonstrated in both the Marchi and the Weigert preparations. There is no evidence either in the literature or in a study of our own cases that an actual inflammatory process takes place in the peripheral nerves. The nerve endings in the muscles in 3 of our cases were studied (Cases 1, 2 and 3) by the Larvelle-Renmont technic. This showed that even in the muscle areas where there is extensive degeneration and atrophy the nerve endings are well preserved.

Summary

1. Five cases of dermatomyositis are reported, 2 of which have complete postmortem examinations. The literature is reviewed and the etiology and clinical course of the disease are discussed.

2. The pathology is discussed. The skin changes are not specific for this disease. The cardinal skin changes are atrophy of the epidermis, flattening of the dermal papillae, edema and fibrosis of the derma and lymphocytic infiltration, especially in the perivascular spaces and around the sweat glands and hair follicles. The muscle changes are of a non-specific non-inflammatory nature and consist for the greater part of focal lymphocytic infiltration and degeneration.

3. The findings in the peripheral and central nervous systems are described. The nerve endings are found to be uninvolved. Changes in the peripheral nerves are described which are apparently secondary to the muscle lesions.

4. An attempt is made to simplify the nomenclature of the disease. It is suggested that such terms as "acute hemorrhagic polymyositis," "dermato-mucoso-myositis," "neuromyositis" and "angiomyositis" are unnecessary as all the lesions which these terms stress are found in "dermatomyositis."

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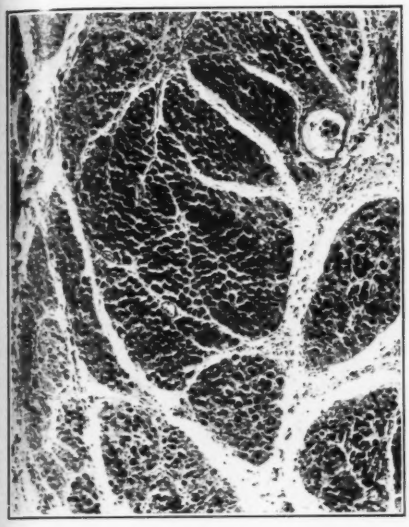
DESCRIPTION OF PLATES

PLATE 113

FIG. 1. Section of the pectoralis minor showing moderate muscle involvement together with many normal muscle fibers. × 45.

FIG. 2. Another section from the same muscle in which muscle degeneration and atrophy are more marked. × 45.

FIG. 3. Section of the pectoralis major illustrating lymphocytic infiltration of the vessel walls and deposition of hyaline material within the media, together with lymphocytes within a lymphatic channel. × 175.



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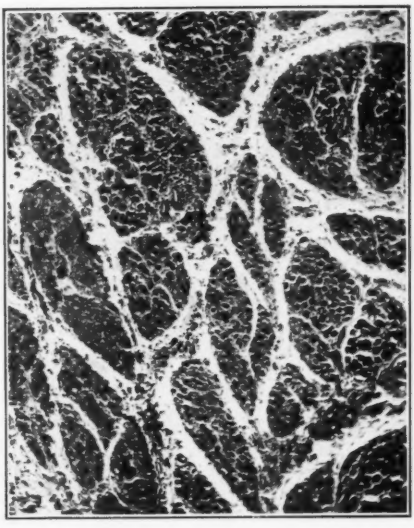

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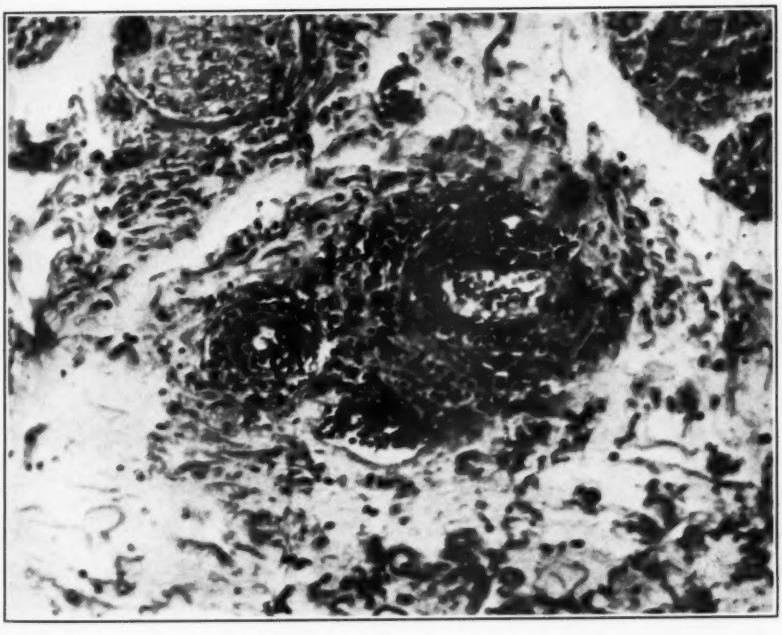


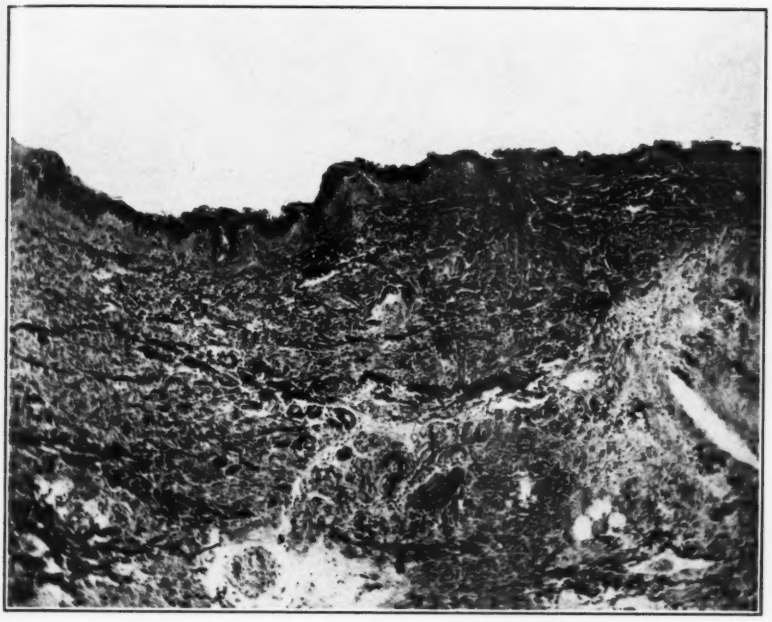
3

Kinney and Maher Dermatomyositis

PLATE 114

FIG. 4. Skin from the right knee demonstrating the transition between normal epidermis and an area of atrophy of the epidermis. × 55.

FIG. 5. Partial obliteration of a blood vessel in a necrotic area from the derma of the right knee showing deposition of amorphous material within the lumen. × 250.



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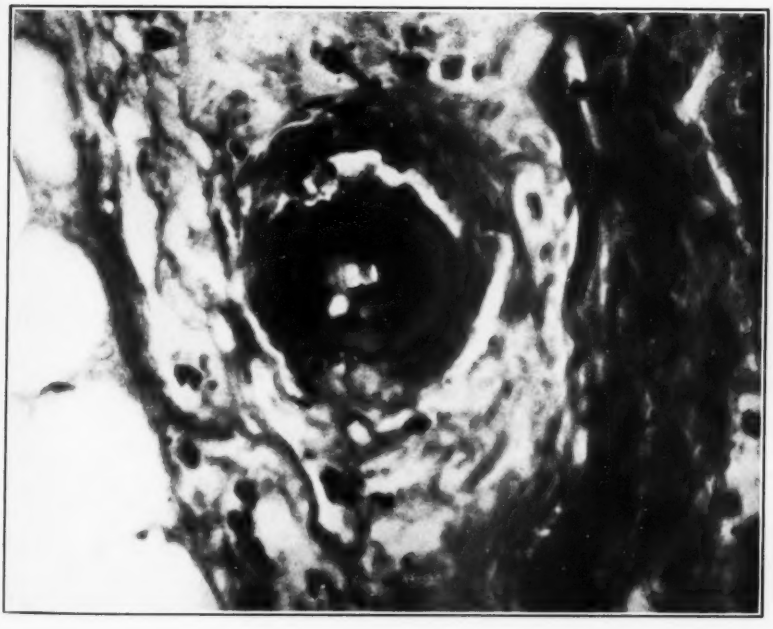


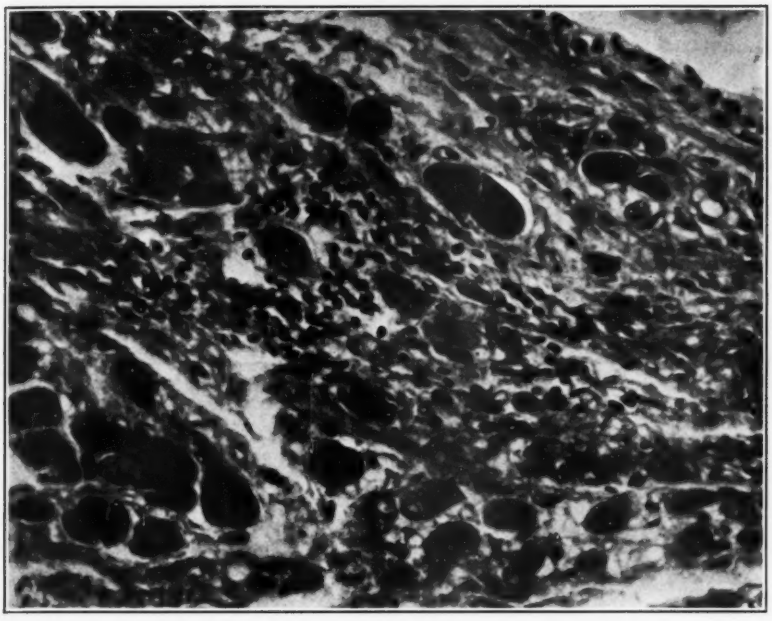
5

Kinney and Maher Dermatomyositis

PLATE 115

FIG. 6. Cardiac muscle illustrating degeneration and atrophy of muscle fibers with lymphocytic infiltration between muscle fibers. × 250.

FIG. 7. Portion of the diaphragm showing lymphocytic infiltration between the muscle fibers. × 95.



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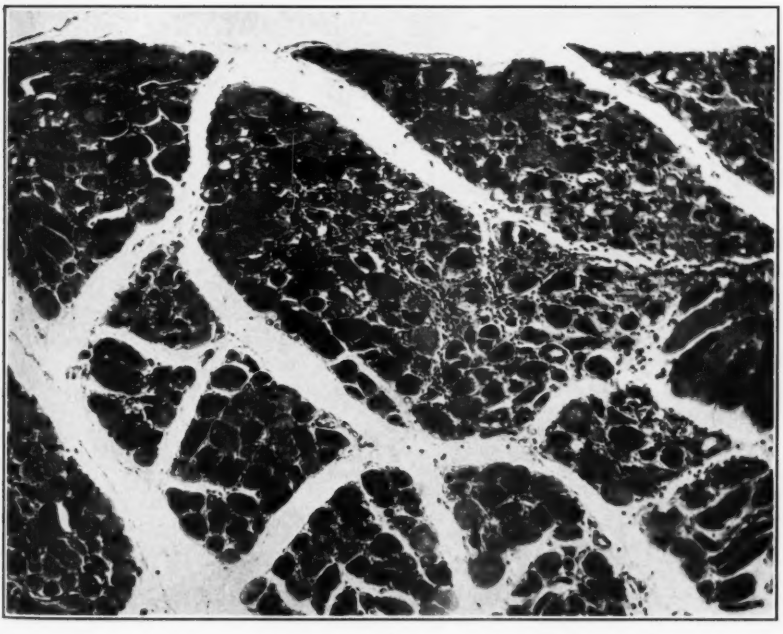
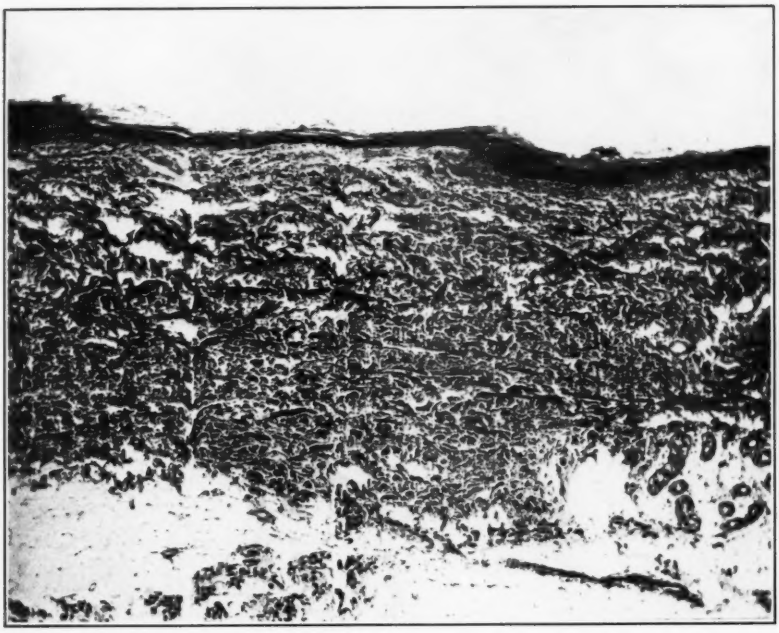


7

PLATE 116

FIG. 8. Skin from the pubic region illustrating the extreme thinning of the epidermis. × 55.

FIG. 9. A portion of a cranial nerve nucleus with a perivascular cuff of lymphocytes and interstitial lymphocytic infiltration. The neurons are injured and are surrounded by glial proliferation. × 55.



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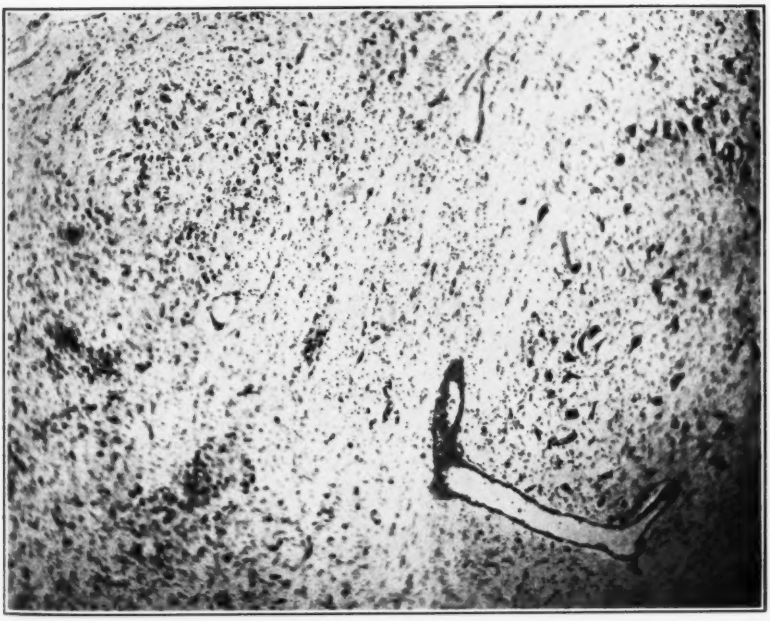

9

EOSINOPHILIC GRANULOMA OF BONE *

With Report of a Case

Louis Lichtenstein, M.D., and Henry L. Jaffe, M.D.

(*From the Laboratory Division, Hospital for Joint Diseases, New York City*)

This report concerns a peculiar lesion of bone that has apparently escaped general recognition. It is true that a few pertinent cases have been described in the literature, under one heading or another. Thus, Finzi [1] describes under the heading of "myeloma with prevalence of eosinophile cells," a case in which the lesion was in the frontal bone of a boy of 15 years. Mignon,[2] under the heading of "a granulation tumor of the frontal bone," gives a clinical and roentgenographic description of another case which may well be one in point but in which adequate anatomical confirmation is lacking. In this case the lesion also was in the frontal bone, and the patient was a boy of 12 years. Schairer [3] described 2 cases of a benign disease of the child skull (osteomyelitis with eosinophil reaction). In each of these cases the lesion was in a parietal bone, and in each again the patients were boys, of whom one was 9 and the other 10 years old. Schairer makes no mention of Finzi's case, and Mignon makes none either. Furthermore, these various case reports do not leave one with the impression of a clearly defined entity such as we consider the lesion to represent.

This lesion is designated "eosinophilic granuloma of bone" for reasons to be indicated presently. Our experience includes 1 case treated during 1938 in our hospital, and followed since, in which the lesion was in the femur of a 4 year old girl. We have also seen material from a case treated at another hospital in which the lesion was in the frontal bone of a 21 year old man, and from another case, in still a different hospital, with involvement of a rib of an 11 year old boy. These last 2 cases, seen in consultation, supplement the information gleaned from our own case. It is the latter case, however, that substantially provides the basis for the ensuing discussion, which will also take cognizance of the cases already

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reported under various headings in the literature and previously mentioned.

Eosinophilic granuloma of bone presents itself as a rather well localized, single lesion starting in the medullary cavity and tending to erode, expand and perforate the cortex in the bony site affected. When first observed the lesion may already have perforated the cortex and extended into the neighboring soft tissues. Indeed, the roentgenographic and clinical findings may lead one to suspect the presence of a malignant bone tumor. Surgical exploration shows that the affected portion of the bone has been extensively replaced by a more or less soft, yellowish or brownish tissue. In its microscopic appearance the latter is characterized essentially by the presence of compacted, tumor-like aggregates of large phagocytic cells, with conspicuous collections of eosinophilic leukocytes interspersed.

The interpretation of this picture is perplexing, for it does not suggest any classified disease of bone, either inflammatory or neoplastic, with which we are familiar. It seems to us that the condition in question may be regarded provisionally as a peculiar, inflammatory granulomatous lesion of indeterminate nature. We appreciate that the term proposed for this lesion is not altogether above criticism. It is true that we know nothing of the etiology of this granuloma. Nevertheless, the name we have given to this lesion at least serves to call attention to the heavy infiltration with eosinophilic leukocytes which is generally such a prominent and consistent histological feature.

Report of Case

Clinical History: The patient was a white female child, 4 years of age, who was admitted to the hospital on Nov. 7, 1938, because of pain and swelling of the left thigh of about 2 weeks duration. The past history was not significant except for the occurrence of whooping cough in 1936, and otitis media in 1937. The family history was irrelevant except for contact with two aunts who had had pulmonary tuberculosis.

Three weeks before admission the child began to cry at night and complain of pain and discomfort. At this time also the mother noted a limp. The following week the child began to localize the pain in the region of the left thigh and became plaintive and subdued, whereas formerly she had been quite active. During the week prior to admission these symptoms became more pronounced. No history of trauma was elicited.

Physical Examination: This revealed a fairly well nourished and well developed child who appeared ill and listless. The temperature ranged between

99 and 100.6° F. by rectum. The patient walked with a slight limp, holding the left leg abducted 10–15°. There was a slight, ill-defined swelling of the upper portion of the left thigh, the circumference of which was about ⅝ inches greater than that of the right thigh. Motion of the left hip was somewhat limited in all directions. There was definite tenderness over the anterior and lateral portions of the thigh, 3 inches below Poupart's ligament, corresponding to the area of maximal swelling. The superficial veins in the left groin appeared slightly dilated. The left inguinal lymph nodes were not enlarged.

Laboratory Examinations: A blood count gave the following values: hemoglobin 12.2 gm. (Sahli), red blood cells 4.0 million, and white blood cells 9200, with a differential count of polymorphonuclears 49 per cent, lymphocytes 45 per cent, eosinophils 4 per cent, basophils 1 per cent, and monocytes 1 per cent. Serum chemical estimations, including those of protein, sugar, nonprotein nitrogen and uric acid, were within the normal range. The Kahn test was negative.

Roentgenographs of the left femur (Fig. 1) disclosed an expanded, somewhat fusiform area of rarefaction within the proximal third of the shaft of the femur. This medullary defect extended from the intertrochanteric region down the shaft for a distance of approximately 3 inches. There was evidence of periosteal new bone deposition, which could be traced downward below the lesion to the junction of the proximal and middle thirds of the femur. The cortex at the site of the lesion was distinctly thinned in consequence of endosteal erosion, and appeared to be broken through in its proximal portion. There was suggestive trabeculation within the rarefied osseous defect. The X-ray picture was quite plausibly interpreted by the roentgenologist as probably indicating the presence of a tumor within the upper end of the femur. Roentgenographs of the other bones of the left lower extremity and of both upper extremities failed to disclose any other lesions.

Operation: A definite preoperative clinical diagnosis was not made, but the following tentative opinions were ventured: osteomyelitis, tuberculous osteitis and Ewing's tumor.

Operation was performed on Nov. 9, 1938. A 4 inch incision was made just below the trochanter on the lateral side of the femur. The periosteum was found congested and the cortex thinned. A cortical window was removed, exposing a cavity in the bone containing approximately 10–15 cc. of blood-tinged fluid and lined by a soft yellowish tissue which was thoroughly curetted. The wound was then closed tightly, without drainage.

Smears from the operative field revealed many polymorphonuclear leukocytes and a few lymphocytes, but no bacteria. Cultures of the swabbings were sterile after 48 hours. A guinea pig inoculated with tissue from the lesion showed no evidence of tuberculosis after 10 weeks.

Course of Illness: Two weeks after operation, because of the interesting picture revealed by the pathological examination of the lesion, the surgeon was advised to perform a sternal bone marrow biopsy. Microscopic study of the tissue thus obtained showed an increase in the proportion of eosinophilic myelocytes and young eosinophilic granulocytes. These cells constituted almost 15 per cent of the marrow cells. The neutrophilic polymorphonuclear leukocytes were not increased in number, nor did the other cellular elements of the sternal bone marrow appear to be significantly altered.

The postoperative course during the stay in the hospital was uneventful and the wound healed per primam. The patient was discharged from the hospital on Dec. 3, 1938.

On empirical grounds the child was given postoperative deep roentgen therapy. Subsequent roentgenographs suggested progressive filling in of the curetted bone area in the femur. When the child was last seen, about 1 year after operation, she appeared well and active, and was symptom-free. A differential white blood count done at that time still showed an eosinophilia of 5 per cent, however. This is undoubtedly an interesting observation, but its interpretation leads one into speculation.

Pathological Examination of Material from the Femur

The surgical specimen consisted of numerous fragments of soft yellowish green tissue curettings from the rarefied area in the upper end of the femur.

Sections stained with hematoxylin and eosin showed a striking histological picture, unique in our experience at that time. The non-osseous material was composed of a cellular tissue, densely infiltrated by large nests, indeed by whole sheet-like masses, of eosinophilic polymorphonuclear leukocytes which stood out vividly in the hematoxylin and eosin stain (Fig. 2). Interspersed between the collections of eosinophils were compacted, tumor-like aggregates of cells of fairly uniform type, with large, pale, oval or kidney shaped nuclei (Fig. 3). Their cytoplasm was pale staining and rather indistinctly outlined. Many of these cells had two or more nuclei. In addition there were an appreciable number of larger multinuclear (giant) cells present, not resembling megakaryocytes or osteoclasts, which had apparently resulted from coalescence of the basic proliferating stromal cells (Fig. 4). The latter had some superficial resemblance at first sight to myeloid tumor cells. Indeed, our initial impression was that the lesion might be an eosinophilic myelocytoma. Closer inspection, however, revealed that many of the cells in question contained phagocytozed eosinophils and erythrocytes, as well as eosinophilic and reddish brown granules. This feature was particularly well demonstrated by the Giemsa stain. The intracellular eosinophilic granules apparently resulted from disintegrated phagocytozed eosinophilic leukocytes, while the more brownish granules were identified as iron-containing pigment, presumably from broken-down red blood cells. Clearly then, the closely packed large cells which are so prominent a feature of the lesion represent large

phagocytes (Fig. 5). Whether these represent wandering macrophages, or histiocytes derived from the fixed tissue reticulum of the bone marrow, is difficult to decide.

In addition to the large aggregates of eosinophils there were present also plasma cells, small lymphocytes and neutrophilic polymorphonuclear leukocytes. However, there was no suggestion of abscess formation or other indication of a suppurative infection. In this connection it may also be pointed out that cultures for bacteria remained sterile. The lesion was moderately vascular, being permeated by capillaries and other thin walled blood vessels, and there were areas of extravasation and hemorrhage. The supporting connective tissue framework was delicate.

Sections of the osseous fragments showed the intertrabecular spaces to be infiltrated by the same type of tissue. The cortical bone was porotified but not at all necrotic. The enlarged vessel spaces contained fibrous tissue which was infiltrated by small round cells and a few leukocytes.

The condition was difficult to diagnose. We felt that the lesion represented a peculiar granuloma of bone of undetermined etiology. Our final diagnosis was eosinophilic granuloma of femur of undetermined etiology (possibly a virus granuloma).

Discussion

After observing this case, and appreciating its uniqueness, we were on the alert for other similar cases. In September, 1939, one of us (H. L. J.) was asked to see a case in consultation, which turned out to be pertinent. The patient was a boy 11 years of age who for several weeks had noted a swelling over one of his ribs. The X-ray picture of the lesion had been misread by everyone who saw the plate as indicating the presence of a malignant tumor, probably a Ewing's tumor.*

* This patient was seen through the courtesy of Dr. Murray H. Bass and Dr. Harold Neuhof, and he was operated upon at the Mount Sinai Hospital, New York City. A detailed written opinion on this case was submitted, drawing upon our experience with the femur lesion already discussed. This opinion included the statement that "certain features of the lesion suggest that it is some sort of a granuloma, but it does not fit into any of the well known granuloma categories." In the July issue of *The American Journal of Pathology* there appeared an article by Otani and Ehrlich entitled, "Solitary granuloma of bone, simulating primary neoplasm," which deals with a number of instances of the lesion in question here, but builds its discussion particularly upon this case. We are glad to know that

Subsequently material from a lesion in the frontal bone of a calvarium was shown to us, which was said to have broken through the inner table of the skull into the dura. This lesion had been surgically resected and the patient, a young man about 21 years of age, had been given postoperative roentgen therapy on the premise that he had a bone tumor. And yet, fully 6 years later, the patient was known to be alive and well. Examination of the microscopic sections showed that the lesion in question also resembled the one we had seen in the femur.†

With these 3 cases in mind, and appreciating that they constituted a lesion *sui generis*, we began searching in the medical literature for other similar cases. We found the 3 reports (covering 4 cases) already mentioned. These are simply case reports and present no detailed discussion of the lesion in question. It is interesting to note that in all of these cases the lesion was in the calvarium and had perforated one or both tables of the skull. While we cannot agree with the interpretations of the underlying pathology of the lesion implied in the titles chosen by the authors, their descriptions parallel our own findings rather closely. In connection with the microscopic lesions there are some slight histological variations from case to case, for instance in the degree of infiltration by eosinophilic leukocytes, the prominence of the histiocytic cells and the extent of phagocytosis, the number of giant multinuclear cells, and the vascularity of the lesion as a whole. However, the basic histological pattern remains essentially the same in all cases.

For reasons that have already been made clear in connection with the description of our pathological findings, we feel that the lesion in question represents neither a myeloma nor an osteo-

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Drs. Otani and Ehrlich have come to the same conclusion in regard to the latter.

We also wish to note in this connection the report on Case 26302 from the Case Records of the Massachusetts General Hospital, in *The New England Journal of Medicine*, July 25, 1940, **223**, 149. On the basis of the pathological examination this case is reported as an instance of "eosinophilic granuloma of bone." In discussing the pathology of the lesion Dr. Tracy B. Mallory accepts our designation and interpretation of it as a specific and distinctive one.

† This case was seen through the courtesy of Dr. Herman Bolker. Since submitting our manuscript for publication we have had the opportunity of studying material from still another case which he kindly put at our disposal. In this case the patient was an infant 10 months old, in whom the lesion involved over one-third of the distal part of a radial shaft.

myelitis, as others [1, 3] have suggested, but rather some type of a granuloma. However, it does not fit into any of the recognized granuloma categories. We venture the suggestion that the lesion may conceivably represent a virus granuloma. We regret that the idea occurred to us after the surgical specimen from our principal case had been fixed in formalin, but we propose, in future, to preserve fresh tissue in 50 per cent glycerin for animal inoculation.

The finding of an increased proportion of eosinophilic myelocytes and young eosinophilic granulocytes in the sternal bone marrow in the case presented here suggests that there may be a systemic response to the etiological agent or factor. This tendency to active eosinophilic myelopoesis is also reflected by the finding in our case, and likewise in the reported cases,[1, 2, 3] of a significant eosinophilia in the blood smears, ranging between 4 and 10 per cent, and not ascribable to any other demonstrable cause.

In regard to the differential diagnosis, it should be pointed out that certain clinical features may lead one to suspect the presence of eosinophilic granuloma of bone even before surgical intervention is attempted. Among these considerations are the occurrence of the lesion in a child or a very young adult, a short history of painful localized swelling of only several weeks duration, roentgenographic evidence of a rarefied and destructive bone lesion (notably in the calvarium, but possibly in a rib or in a long bone), with perforation of the cortex and perhaps a palpable soft tissue mass overlying the affected bone. The finding of a slight or moderate eosinophilia (4–10 per cent) in the differential white blood count may also be helpful. If an aspiration needle puncture is done, examination of the tissue may reveal the presence of numerous eosinophilic leukocytes.

However, surgical exploration and pathological examination are really essential in establishing the definitive diagnosis. The microscopic sections readily enable one to rule out all types of ordinary malignant bone tumors, including Ewing's tumor, reticulum cell sarcoma, and spindle cell or osteolytic osteogenic sarcoma. Another condition, in particular, with which the lesion may conceivably be confused, namely solitary myeloma, may likewise be excluded by histological examination. Other osseous lesions from which the condition in question is to be differentiated need only be mentioned, *e.g.* the bone lesions of aggressive myeloid leu-

kemia, of Hodgkin's and of Hand-Schüller-Christian's disease, bone cyst, and posttraumatic rarefaction.[4]

Finally, one must consider the possible relationship of the condition in question to so-called eosinophilic leukemia or the clinical syndrome discussed by Bass [5, 6] under the heading, "Unusual eosinophilia with splenomegaly (eosinophilic leukemia?) in a child." This question is naturally suggested by the relatively high incidence in young individuals, and by the finding of active eosinophilic myelopoiesis and of eosinophilia in the circulating blood in cases of eosinophilic granuloma of bone. There are many essential features, however, pertaining to the clinical entity of so-called eosinophilic leukemia, which are entirely lacking in the condition with which we are dealing, namely a chronic clinical course, splenomegaly, enlargement of lymph nodes, pronounced eosinophilia (30–80 per cent), and persistent leukocytosis often reaching leukemic proportions. Moreover, in none of the case reports discussed by Bass was there any mention of the presence of focal, tumor-like bone lesions.

Treatment and Prognosis

The treatment of choice, as we see it, is wide surgical excision of the lesion. When the lesion occurs in a long bone, or in the calvarium, thorough curettage will suffice. A more radical procedure does not appear to be indicated. In regard to the value of, or necessity for, postoperative roentgen therapy, it should be mentioned that the patients in the foregoing reports did receive such treatment on empirical grounds and made a complete recovery. On the other hand, some of the published case reports previously cited state that satisfactory improvement was noted after surgery alone. This would indicate that postoperative roentgen therapy may not be essential to recovery.

Despite the rapid development of the condition and its seemingly aggressive nature, as reflected by a more or less ominous roentgenographic picture, the prognosis appears to be uniformly good. The postoperative follow-up observations, in all the cases in which this information was available, were gratifying indeed. In every instance complete relief of symptoms was noted and there was roentgenographic evidence of gradual repair of the eroded bone. The period of observation ranged from 7 months to 6 years. It seems fair to conclude that we are dealing with a benign lesion,

and that adequate surgical extirpation, with or without subsequent roentgen therapy, may be expected to effect a clinical cure.

Summary

This paper deals with a peculiar and hitherto not clearly defined lesion of bone which has apparently escaped general recognition. We propose that this lesion be designated "eosinophilic granuloma of bone." It presents itself as a rather well localized, single lesion, starting in the medullary cavity and tending to erode, expand and perforate the cortex in the bone site affected. The latter is found extensively replaced by a more or less soft yellowish or brownish tissue. In its microscopic appearance this tissue is characterized essentially by the presence of compacted, tumor-like aggregates of large phagocytic cells, with conspicuous collections of eosinophilic leukocytes interspersed. Many of these cells contain phagocytozed eosinophils and erythrocytes, as well as eosinophilic granules and brownish iron-containing particles. Some of them have two or more nuclei, and there is also a scattering of giant multinuclear cells.

We venture the suggestion that the lesion may conceivably represent a virus granuloma. The finding of a significantly increased proportion of eosinophilic myelocytes and young eosinophilic granulocytes in the sternal bone marrow in our case suggests a systemic response to the etiological factor. This tendency to active eosinophilic myelopoesis is also reflected in the finding of eosinophilia, usually ranging between 4 and 10 per cent and not ascribable to any other demonstrable cause.

The condition seems to have a predilection for children and very young adults, especially males. It has been noted most commonly in the calvarium. It pursues a rapid clinical course characterized by painful swelling and a tendency to pathological fracture or perforation of the affected bone. The duration of symptoms before the patient seeks treatment may be no more than a few weeks. Despite the rapid development of the condition and its seemingly aggressive nature, as reflected by a more or less ominous roentgenographic picture, the prognosis appears to be uniformly favorable. Our experience, and that of others, indicates that adequate surgical extirpation of the lesion, with or without subsequent roentgen therapy, may reasonably be expected to effect a clinical cure.

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DESCRIPTION OF PLATES

PLATE 117

FIG. 1. Roentgenograph of the left femur showing an expanded area of rarefaction within the proximal third of the shaft. Note the evidence of periosteal new bone deposition, extensive endosteal erosion and perforation of the cortex.

FIG. 2. Microphotograph of the lesion in the femur showing large focal collections of eosinophilic leukocytes. The deeply staining clumped cells are all eosinophils. × 150.

FIG. 3. Higher magnification showing eosinophilic polymorphonuclear leukocytes dispersed among large phagocytic cells. The leukocytes containing large granules are eosinophils. × 475.

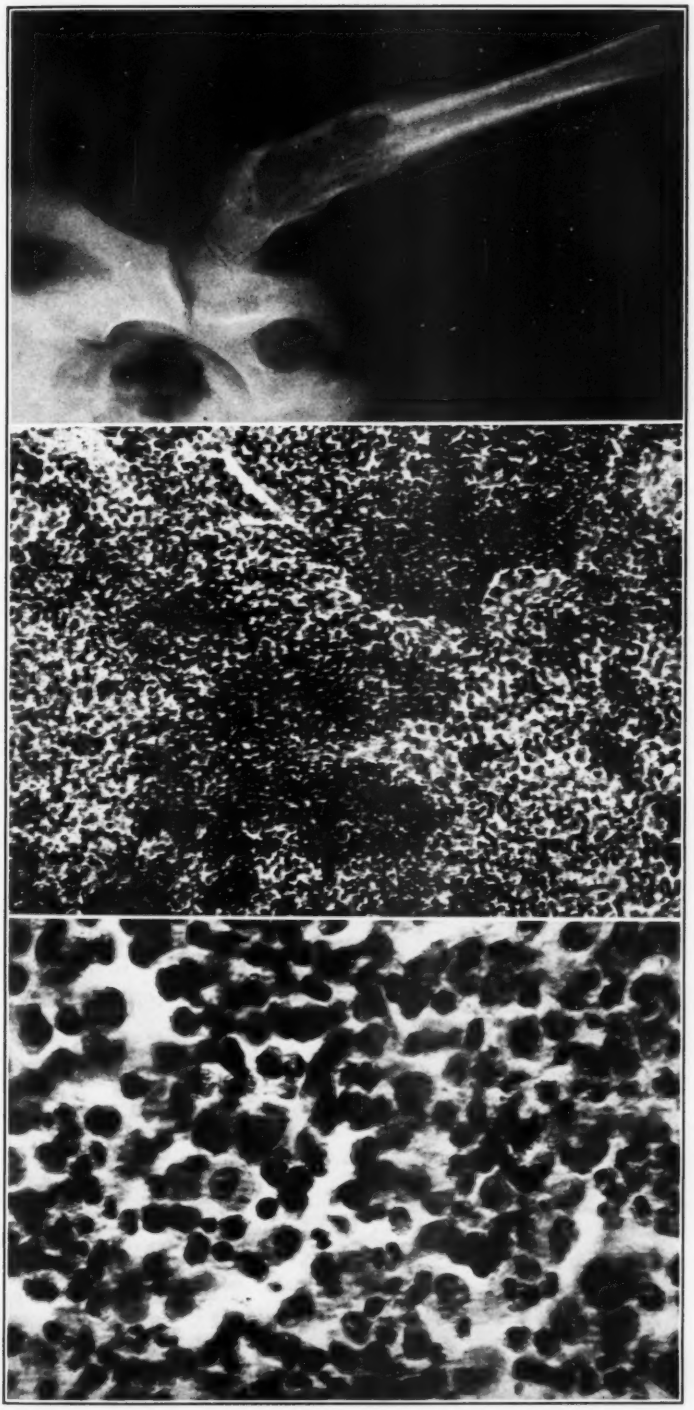

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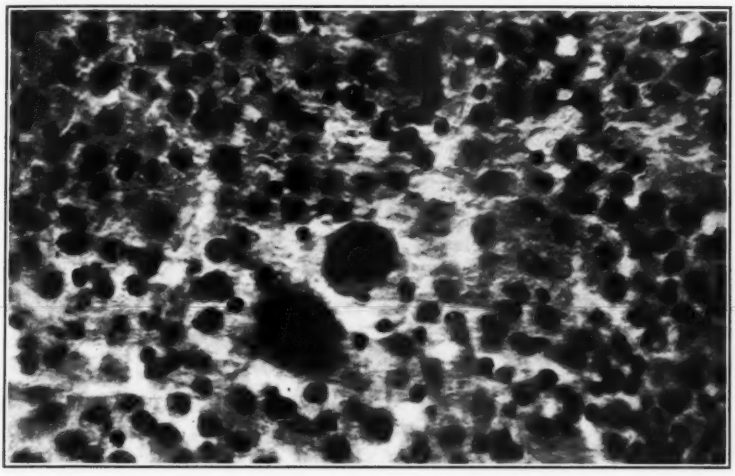
PLATE 118

FIG. 4. Microphotograph of the lesion in the femur showing a number of large multinuclear (giant) cells. Note that they resemble neither megakaryocytes nor osteoclasts. × 350.

FIG. 5. Higher magnification showing a compacted tumor-like aggregation of large phagocytic cells. Note that many of them have more than one nucleus. Some of these cells contain phagocytozed eosinophils and erythrocytes, as well as eosinophilic granules and iron-containing particles. × 700.



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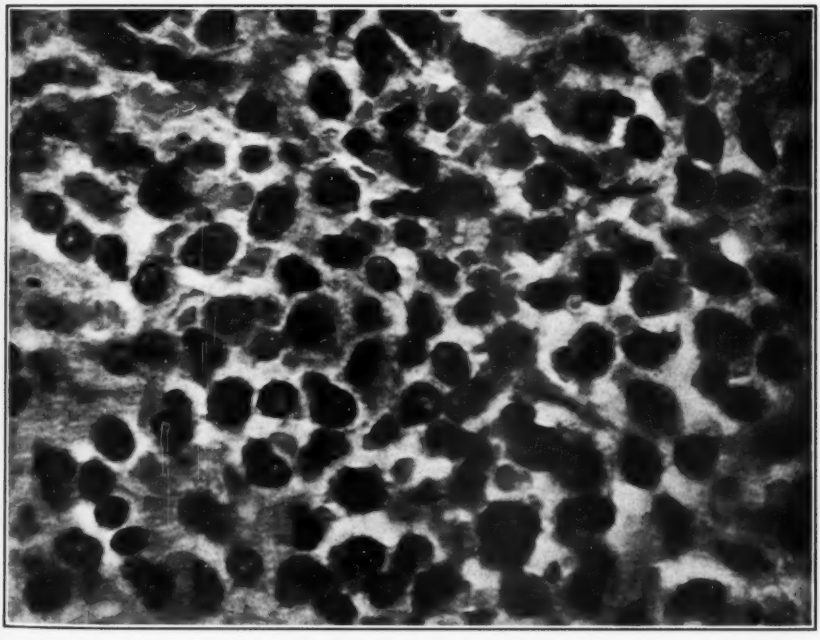

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Lichtenstein and Jaffe Eosinophilic Granuloma of Bone

COMPARATIVE EXPERIMENTAL STUDIES OF 200 KILOVOLT AND 1000 KILOVOLT ROENTGEN RAYS *

I. The Biological Effects on the Epiphysis of the Albino Rat

Edward A. Gall, M.D., James R. Lingley, M.D., and John A. Hilcken

(From the Laboratory of Pathology and Bacteriology, and the Department of Roentgenology, Massachusetts General Hospital, Boston, Mass.)

The imminence of extensive clinical application of supervoltage roentgen rays creates a distinct need for the appreciation of the effects produced by this new source of radiation. Up to the present time there have been very few controlled observations on microscopic changes in mammalian tissues produced by different types of roentgen rays. It is the dual purpose of this study therefore to establish first the effects in albino rats of graded amounts of irradiation produced by the more commonly available high voltage apparatus (200 kv.), and secondly to compare these changes with those developing following irradiation with similar amounts of rays produced by the supervoltage machine (1000 kv.).

It is appreciated that observations made on lower animals may not be unequivocally interpreted in terms of expected effects on man. It must be admitted, however, that such changes as are noted are necessarily more accurate biological indices of the reaction of tissues than methods entailing the use of water and wood phantoms with drosophila eggs, tadpole's tail, and so on.

Methods

Ninety-five albino rats of the same strain were employed as experimental subjects. These were roughly evenly divided as to sex and were all apparently the same age. They were obtained shortly after weaning and were not exposed to treatment until they had been kept on the laboratory regimen for at least 1 week.

* The expenses of this study were defrayed in part by a grant from the Biological Engineering Fund of the Massachusetts Institute of Technology, and in part by a grant from the Infantile Paralysis Research Fund of the National Foundation for Infantile Paralysis, Inc.

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At the time of irradiation the rats were 50 to 54 days of age. The regimen consisted of the Sherman diet BMS recommended by Russell,[1] the composition of which was ground whole wheat 60 per cent, milk powder 30 per cent, meat scraps 10 per cent, and sodium chloride 2 per cent by weight of the wheat. Water was administered *ad libitum*. The rats were kept in the Nutrition Animal Room at the Massachusetts Institute of Technology. They were housed in open meshed wire cages suspended over sawdust-filled trays. Except for a transient period immediately succeeding the administration of higher doses of roentgen rays, all of the animals flourished and gained weight. Only 2 rats succumbed spontaneously, 1 of intercurrent infection and the other presumably because of overexposure of the abdomen to a large amount of irradiation.

As shown in Table I, the animals were divided into two major groups; Group I for treatment with the high voltage, and Group II receiving irradiation from the supervoltage machines. Each of the main groups was divided into six smaller subgroups, one a control and the remainder receiving in a single dose 600 r, 1200 r, 1800 r, 2400 r and 3000 r respectively over the lateral aspect of the right thigh. The animals were sacrificed by decapitation at the periods denoted in Table I from 1 week to 5 months after irradiation.

The qualities of the two machines used were as follows:

Group I Picker-Waite Machine *		Group II Van der Graaf Generator **
200,000 volts	Constant potential	1,000,000 volts
20 cm.	Distance	50 cm.
20 milliamp.	Current	0.85 milliamp.
0.25 mm. copper, 1.0 mm. aluminum	Filtration	3.0 mm. lead, 8.0 mm. copper
0.75 mm.	Copper half value layer	10.5 mm.
300 r/min.	Intensity	133 r/min.
10 cm.	Cone	10 cm.

Dosages were expressed in roentgens measured in air with the Victoreen dosimeter. The dosimeter was checked periodically against two others.

The animals were immobilized with their hind extremities widely

* At the Baker Memorial, Massachusetts General Hospital.
** At the Collis P. Huntington Memorial Hospital.

TABLE I

Tabulation of Experimental Data

Group I (200 kv.)													Group II (1000 kv.)											
Subgroup	Number of rats	Amount of irradiation	Sacrificed (weeks after irradiation)											Subgroup	Number of rats	Amount of irradiation	Sacrificed (weeks after irradiation)										
			1	2	3	4	5	6	7	8	9	11	13				1	2	3	4	5	6	7	9	11	16	19
A	10	None	+ + + +	+	+	+			+			+ *	+	A	8	None	+ + +	+	+	+				+			+
B	7	600 r	+	+	+	+	+			+		+		B	7	600 r	+	+	+		+			+		+	+
C	6	1200 r	+	+	+	+			+			+		C	8	1200 r	+	+		+		+	+	+	+	+	
D	7	1800 r	+	+	+	+	+		+				+	D	10	1800 r	+	+	+	+	+		+	+	+	+	+
E	6	2400 r	+	+	+	+		+					+	E	10	2400 r	+	+	+	+	+	+	+	+	+	+	
F	6	3000 r	+	+	+	+				+	+			F	10	3000 r	+	+ + **	+		+	+	+	+	+	+	
Total	42													Total	53												

* Died of intercurrent infection.
** Died of overexposure of abdomen.

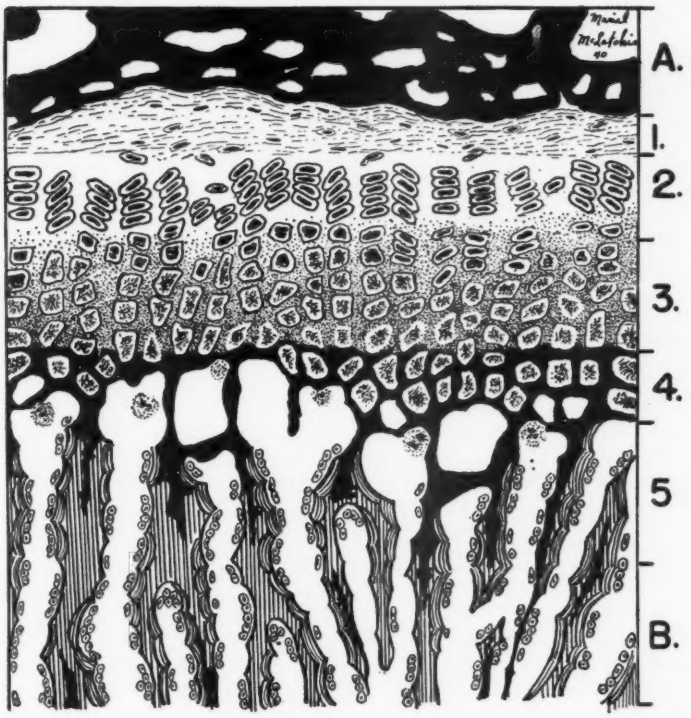
abducted on a three ply, ⅜ inch plywood Y-shaped board by snug swathing with adhesive tape and an ace bandage. Treatment was given in a single dose to the lateral surface of the right thigh, which was placed directly beneath the treatment cone, the field including the knee joint. Care was taken to exclude as much of the abdomen as possible.

Following sacrifice the right femur was disarticulated at both the acetabulum and knee joint and dissected free of muscle and integument. The lower half was then split longitudinally between the condyles and both fragments were fixed in Zenker's fluid plus 5 per cent acetic acid. The acetic acid satisfactorily decalcified the bone specimens in all except the very oldest animals and these were further decalcified in nitric acid-alcohol solution for 6 to 12 hours. Specimens were all embedded in paraffin and stained with phloxine-methylene blue.

Definition of Terms

The nomenclature utilized by the authors is similar to that given by Dodds and Cameron.[2] Text-figure 1, representative of the epiphyseal region in the growing rat, demonstrates diagrammatically the various zones concerned with growth. The horizontal disc of hyaline epiphyseal cartilage lies between the bony plate of the center of ossification (A) and the metaphysis (B). The cartilage is subdivided roughly into five zones. Zone 1, contiguous with the epiphyseal bone, consists of reserve or "mother" cells comprising an area analogous to the active or germinal layer of the perichondrium. The cellular elements are small, deeply staining and flattened, and are surrounded by a matrix resembling connective tissue as much as it does cartilage. The cells are not unlike fibroblasts but are encompassed by ill-defined lacunae. Zone 2, immediately proximate on the shaft side, has been termed the zone of cell multiplication in columns. In this region chondroblasts now surrounded by well formed lacunae are arranged in parallel columns in the direction of the long axis of the bone. Intervening between the columns is a homogeneous basophilic matrix of hyaline cartilage. In this region growth is initiated by longitudinal mitotic proliferation. Zone 3 represents the area of intrinsic cellular growth; further increment in length is produced here by an increase in the size of individual chondroblasts without additional multiplication of cells. In Zone 4, the cartilage cells

and lacunae having reached maximum size, the solidity of cartilage is enhanced by the deposition of mineral salts (calcification) in the hyaline matrix. In Zone 5, which consists of calcified primary trabeculae with intervening primary marrow, there is removal of



TEXT-FIG. 1. Diagrammatic representation of the histological structure of the epiphysis of the long bone of the albino rat. See text for descriptive interpretation. Reproduced with modifications through the courtesy of Dr. T. H. Ingalls from "The Roentgenologic Details of Bone Growth." To be published.

effete chondroblasts and excess calcified trabeculae. In this region osteoblasts are superimposed on the surface of those primary trabeculae that have not been resorbed and proceed to lay down osteoid, mineralization of which produces true bone. Intervening tongues of vascular and cellular substance constitute the primary metaphyseal marrow.

Observations

Perthes in 1903 [3] first observed the growth-inhibiting qualities of roentgen rays. Adequate subsequent confirmation [4–9] has established the marked susceptibility of the epiphyseal cartilage of growing bone to the effects of irradiation. Attention, in this particular study, has been directed at the epicondylar epiphysis of the femur, in which active growth is known to persist unretarded throughout the age limits of this experiment.[10] Any deviation, therefore, from the normal structure as described by Dodds and Cameron [2] and demonstrated by our control group (Fig. 1) was presumed to represent the effects of irradiation. Frequent observations of the contralateral untreated extremities of the same animals offered additional control material, although it was not possible to screen this area entirely from the supervoltage rays.

Since none of the doses utilized in this study failed to produce significant histological modification in the epiphyseal cartilage, this structure may be considered to be an extremely sensitive indicator of the effect of irradiation. It was reasonably evident that the degenerative lesions noted in the epiphyseal cartilage were peculiar to this structure, since the articular cartilage included within the same sections revealed little or no damage. In fact, such lesions as were apparent in the articular cartilage did not occur until quite late in the course of the experiments and could readily be attributed to the secondary effects of interference with nutrition, damage to the subjacent epiphysis, and the trauma of abnormal weight bearing. Marrow substance in both the metaphysis and the epiphysis was, on the other hand, primarily severely affected by irradiation. Hematopoietic elements reacted in a manner essentially similar to that exhibited in the diaphyseal region and will be dealt with in a separate communication.[11] For purposes of simplicity the injury produced by roentgen rays developed by the 200 kv. machine will be described initially. Comparison with the injurious effects following irradiation from the supervoltage apparatus will then be appended.

200 Kilovolt Roentgen Rays

The grade of severity of epiphyseal damage was found to be directly proportional to the dose as measured in roentgens up to

1800 r, above which point maximal changes prevailed. Such evidence of correlation between the intensity of the treatment and the effects produced has been recorded by others.[4-6]

The earliest evidences of the effect of irradiation appeared within 1 to 2 weeks following all doses used. These were manifested to a varying degree in Zone 2 by swelling of chondroblasts, enlargement of lacunae, irregularity of chondroblast nuclei, increase of intercolumnar cartilaginous matrix with concomitant separation, and slight irregularity of the columns themselves (Fig. 2). During the ensuing week there was a slight but barely perceptible excess of bone deposit on the primary cartilaginous trabeculae in Zone 5. The relative degree of increased osteoblastic activity in this phase was so slight that it might readily have been overlooked were it not for the more advanced processes observed with the higher doses. Dahl [7, 12] has indicated evidence of increased bone formation as early as 3 hours after irradiation. With doses of 600 r and 1200 r there was little progression of the lesion beyond this point, although no retrogression of the damage appeared thereafter. Growth of cartilage continued for a variable period after treatment,[9, 13] but its rate as determined by roentgenograms was obviously impeded.

With dosages of 1800 r to 3000 r there was a marked increase in the severity of reaction after the 3rd week. The degree of severity in these was no longer proportionate to the intensity of irradiation, but was roughly equal in extent for all. Columnar disarrangement became extreme, initially in Zone 2 (Fig. 3) but promptly thereafter in Zones 3 and 4 as well (Fig. 4). In some regions columns were completely destroyed and only a few scattered distorted chondroblasts remained (Fig. 5). Elsewhere there were ragged rows with loss of orientation and widely swollen lacunae which contained either shrunken detached chondroblasts with pyknotic nuclei or which were entirely empty. In Zone 1 primitive cells exhibited only minimal degenerative change but the propensity for proliferative growth and differentiation into mature chondroblasts was not reestablished except in a few unusual instances which will be described below.

Associated with columnar disintegration there was a relative increase in cartilaginous matrix. Despite this apparent increase there was evidence of intrinsic structural defect manifested by

wide variation in staining qualities and a mottled appearance (Fig. 5). This was further intensified by the combination of diminished opacity and coarsening of the normal fibrillar background. Later the fibrillar quality was entirely obscured by transformation of the cartilage into a densely staining block-like substance which fragmented readily on sectioning. In the course of time the cartilaginous disc became flattened and narrowed as the result of the combined effect of intrinsic atrophy, weight-bearing compression, and the absence of replenishment by primitive cells.

Adequate stains for the determination of calcification of cartilage in Zone 4 were not made. Since the staining methods which were utilized were of little value for this purpose, analyses of variations in mineral deposits were not attempted. It was noted, however, that Zone 5, in which tongues of calcified cartilage were normally ensheathed by a thin layer of osteoid (Fig. 1), the slight excess of ossification noted above with small doses of roentgen rays became progressively greater with larger doses. It was observed that the metaphyseal trabeculae were shorter and much broader than normal and it was readily determined that this was the result of continued osteoblastic activity in the absence of the propelling force of growing cartilage which normally displaced the primary trabeculae toward the shaft. Eventually accumulation of bone upon the immobile trabeculae caused obliteration of the primary marrow spaces and ultimate fusion with the production of a horizontal ossified plate immediately subjacent to the epiphyseal cartilage (Figs. 4 and 5). This was not unlike the lesion observed by Dawson [14] in spontaneous "delayed epiphyseal fusion" occurring in older rats.

Osteoblasts were obviously much less affected by roentgen rays than were the epiphyseal chondroblasts. Figures 4 and 5 demonstrate the increasing thickness of superposed knurled bone upon the calcified primary trabeculae. The normally serrated appearance of Zone 5 was lost and the residual unresorbed blue staining cartilage was eventually replaced by eosinophilic osseous tissue. Dodds and Cameron [15] have recently observed in rachitic rats the metaplasia of isolated chondroblasts, persistent within primary trabeculae, into functioning osteoblasts. Albee [16] and others [6, 17] have shown that neither bone growth nor the adequacy of fracture healing were noticeably affected by irradiation if the epiphyses were

screened from the rays. That they were not entirely unaffected, however, may be readily appreciated in view of the fact that the metaphyseal bone in our animals, though dense, was unusually fragile and brittle. Wilkins and Regen [18] have demonstrated a persistent but diminished phosphatase activity in irradiated bone and several investigators [5, 12, 19] have noted the production of dense but brittle bone under similar circumstances. The presence of subepiphyseal osteosclerosis was apparent in roentgenograms as a broad line of increased density (Fig. 6). This line persisted without evidence of resorption throughout the period of the study.

It is believed that once the epiphyseal cartilage has been seriously damaged by roentgen rays there is little evidence of reestablishment of normal growth.[4, 9, 13] Our studies, however, have indicated that spotty regeneration may occur although no return to perfectly normal structure was observed. Wilkins and Regen [18] and Engel [20] have reported some evidence of such recovery. A spontaneous attempt at reestablishment of growth was apparent in a few of our rats in whom profound disturbance of epiphyseal activity had been produced. These manifestations appeared as early as 4 to 6 weeks after treatment, and were characterized by small focal clusters of actively growing chondroblasts in the cartilage immediately adjacent to Zone 1 (Fig. 7). The clusters produced small amounts of cartilage with normal staining qualities but they lacked regular polarity and succeeded in attaining only a vestige of columnar arrangement. The phenomenon was presumed to represent an abortive reactivation of partially degenerated primitive cells. The cell clusters lacking normal vitality and encapsulated by dense non-viable cartilage formed spherical islets incapable of contributing to further growth of the extremity.

In rare instances the injury sustained by one segment of the epiphyseal cartilage was less than that evident elsewhere. In such cases almost complete restoration of activity was observed along as much as one-half of the base of the epiphysis (Fig. 8). There was reappearance of oriented columns of chondroblasts forming normal hyaline matrix and the non-viable cartilage was split from its attachment to the center of ossification in this region and displaced toward the diaphysis. The region of reactivation exhibited normal zoning characteristics and in the space between the newly restored and the residual devitalized cartilage there was evident

all of the elements normally present in Zone 5, such as calcified primary trabeculae, active osteoblasts and hematopoietic marrow. It is reasonable to suppose that extension of this process over a greater portion of the epiphysis might dislodge the entire disc of devitalized cartilage and osteosclerotic bone. Thus, after a period of growth lapse, longitudinal bone increment might again be established. Unfortunately the time limits of these experiments precluded observations over a sufficiently long period to determine whether or not complete regeneration of epiphyseal function was possible. Additional studies for the purpose of clarifying this point are being carried out at present.

1000 Kilovolt Roentgen Rays

The effect of roentgen rays produced by the supervoltage apparatus differed in no very striking manner from the observations

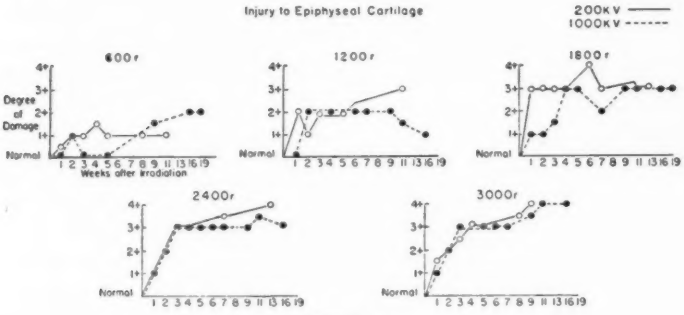


Chart 1

recorded above. The same number of roentgens measured with the Victoreen condenser r-meter caused dose for dose the same ultimate histological changes in the epiphyseal region as were produced by the high voltage apparatus. With 2400 r and 3000 r the histological lesions were exactly similar in both degree and time of appearance (Chart 1). With lower doses (600 to 1800 r), however, although the ultimate effects were the same after exposure to either machine, there was a slight delay in the appearance of histological alteration after supervoltage irradiation. This lag in demonstrable effect, which is shown graphically in Chart 1, was apparent not only with relation to the time at which histo-

logical evidence of injury was first noted, but also to the time at which maximal damage was attained. Stone [21] has commented on retardation in the appearance of the effects of irradiation on the skin following the application of 1000 kv. rays. For practical purposes, however, at least in our experiments with the rat, the effects of equal doses measured as roentgens of the two types of rays are considered essentially similar.

Discussion

It is widely presumed that the greater the degree of proliferative activity, the more susceptible a tissue is to the effects of irradiation. This presumption has in part been confirmed by the studies described above. The fundamental injury produced in the epiphyseal region appeared in the zone of cell multiplication in columns, the other changes, except for those in the marrow substance, following inevitably but distinctly secondarily. It is significant that the articular cartilage in which mitotic division was physiologically minimal exhibited no evidence of primary injury, although subjected to equal amounts of irradiation. In fact, degenerative phenomena were not evident until the destruction of the epiphyseal cartilage had reached a point at which the mechanics of weight bearing and the nutritional supply were obviously deranged. The hypothesis of variation or specificity in degree of radiosensitivity received additional emphasis with the observation of continued osteoblastic function despite the impairment of actual longitudinal growth of bone.

There is general recognition that a greater depth dose is attained with 1000 kv. roentgen rays than with 200 kv. radiation.[22–25] It is necessary to emphasize, however, that since the depth of the lower femoral epiphysis in the 50 day old rat is so small (approximately 0.5 cm.), the effects of differences in penetration of the two qualities of rays would have no influence in the results obtained in this study. A backscatter factor may also be neglected since the backscatter developed from the rat's leg and restraining appliances amounted to less than 2 per cent by measurement for the high voltage rays. It may be reasonably stated, therefore, that our comparisons have been made between equal quantities of radiations as measured by the Victoreen ionization chamber. This being the case, the observations simply prove that equal doses of

roentgen rays derived from high and supervoltage machines produce identical biological effects in the epiphysis of the rat.

The fact that certain structures were only minimally affected by the amounts of irradiation utilized in these experiments and that submaximal doses produced diminution of growth proportionate to the intensity of treatment appeared to be of considerable interest. It occurred to one of our colleagues (Dr. J. S. Barr) that roentgen rays might be utilized clinically in the control of the activity of the epiphysis. Accordingly, experiments are now being performed in an attempt to determine the amount of irradiation which will optimally impede the growth process in the epiphyseal cartilage without causing significant damage to other structures in the path of the X-ray beam.

Summary

Graded amounts of roentgen rays were administered to the thighs and knees of 50 to 54 days old albino rats. Dosage was measured in roentgens in air with the Victoreen dosimeter and ranged from 600 r to 3000 r given in a single dose. The source of irradiation for one group was a 200 kv. machine and for the other an apparatus operated at 1000 kv. constant potential. The effects produced by the two types of irradiation were then compared.

The initial effects of larger doses (2400 to 3000 r) were manifested by complete interruption of physiological activity in the zone of chondroblastic proliferation. Smaller doses (600 to 1800 r) caused submaximal injury to this zone roughly proportionate to the intensity of the irradiation. Subsequently degeneration appeared in the epiphyseal zones distal to the region of primary damage and secondarily the primary spongiosa failed to develop, thus impeding longitudinal growth of the bone. Once suspended, activity was rarely reestablished and then only in a patchy inadequate fashion.

Despite the arrest of cartilaginous function, little or no interference with osteoblastic activity was detected. Deposition of new bone in the subepiphyseal region continued but the defect in epiphyseal activity permitted osteogenesis to progress locally without normal displacment toward the shaft. As a result a plate of knurled sclerotic bone eventually appeared in this region, obliterating the primary marrow spaces and sharply delimiting the

metaphyseal surface of the devitalized cartilage. The bone thus formed, although unusually dense, was apparently much more friable than normal osseous tissue.

There were no detectable differences in the degree of histological modification produced by equal doses in roentgens from either the high or the supervoltage generators. In the lower dose range there was a slight delay of a week or two in the appearance of evidences of injury effected by rays from the 1000 kv. apparatus when compared with those produced by the lower voltage machine. The ultimate effects, however, and the entire sequence of events with the higher dosages were exactly similar regardless of the type of rays employed.

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DESCRIPTION OF PLATES

PLATE 119

FIG. 1. Normal appearance of the epiphysis at the lower end of the femur in a 50 day old albino rat.

FIG. 2. Epiphyseal cartilage 1 week after the administration of irradiation. The columns have become disarranged, the chondroblasts are swollen, and the intercolumnar matrix is mottled and fibrillar.

FIG. 3. Progression of cartilaginous degeneration 2 weeks after irradiation. The columnar arrangement is barely perceptible and there is advanced degeneration of chondroblasts. Slight increase in the amount of subepiphyseal ossification is apparent.

FIG. 4. Twenty-eight days after irradiation. With the exception of Zone 1 immediately beneath the epiphyseal bone, no viable chondroblasts are apparent. The subepiphyseal trabeculae are now obviously thickened and the primary marrow content is depleted.

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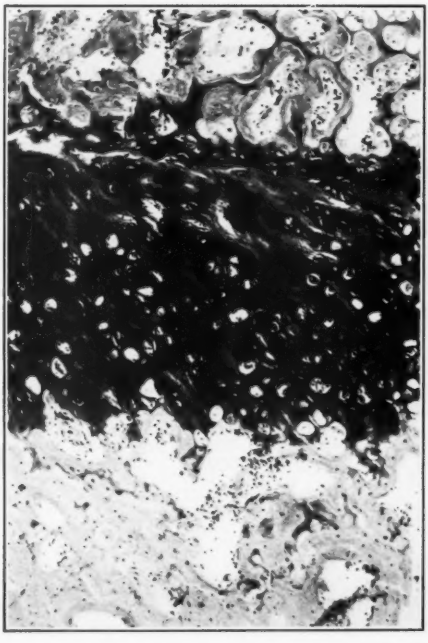

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Gall, Lingley and Hilcken Studies of Roentgen Rays. I

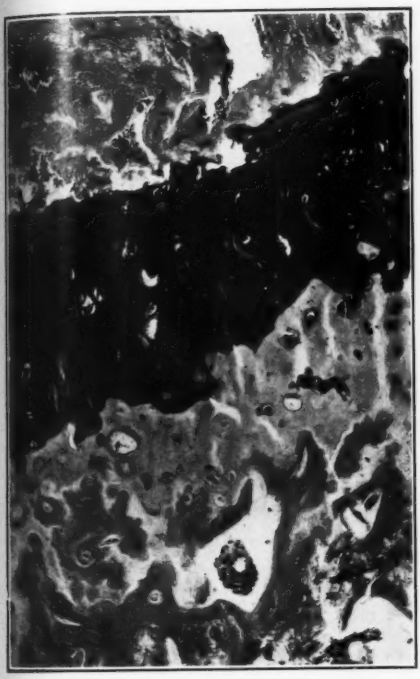
PLATE 120

FIG. 5. Totally non-viable cartilage 60 days after irradiation. Irregularity of staining with deep basophilia of one layer and mottled eosinophilia in a sharply delimited zone below it may be noted. The bony trabeculae have fused to form a subepiphyseal plate.

FIG. 6. A roentgenogram of the disarticulated treated and untreated contralateral extremities of a rat. There is an obvious discrepancy in the size of the femurs and tibias. The treated bones (on the left side of the photograph) are significantly smaller and there is increased radio-opacity in the regions immediately adjacent to the lower epiphysis of the femur and the upper of the tibia. Fifty-six days after irradiation.

FIG. 7. A section exhibiting an abortive attempt at reestablishment of chondroblastic activity. A spherical cluster of viable chondroblasts lies hemmed in by otherwise degenerated cartilage.

FIG. 8. Extensive reactivation of cartilaginous growth. A fairly regular row of chondroblastic columns with normal polarity may be observed along the epiphyseal bony plate. These have begun to form primary trabeculae with intervening marrow substance. The areas of degenerated cartilage and adjacent pathological osteosclerosis have been displaced toward the shaft and may be seen in the lower portion of the illustration.



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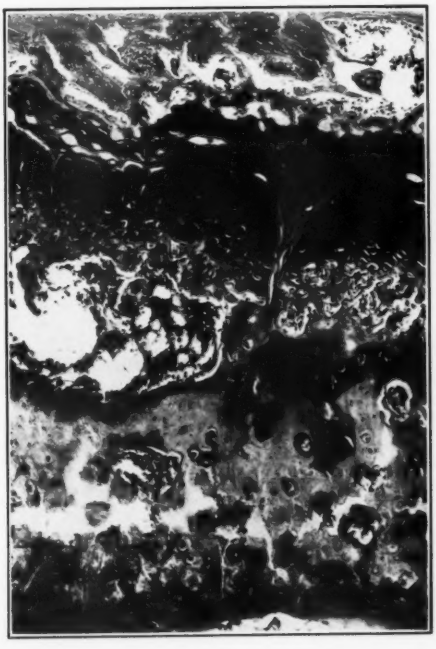

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Studies of Roentgen Rays. I

FAMILIAL OCCURRENCE OF RENAL CARCINOMA IN RHESUS MONKEYS (MACACA MULATTA)*

HERBERT L. RATCLIFFE, SC.D.

(From the Penrose Research Laboratory, Zoological Society of Philadelphia, and the Department of Pathology, University of Pennsylvania, Philadelphia, Pa.)

The incidence of neoplastic disease for the subhuman primates examined postmortem at the Zoological Garden in Philadelphia has been and still is lower than for any other numerically comparable group of mammals. Of 950 of these animals that have been autopsied only 15 developed any sort of tumor, and all except 3 of these were representatives of various species of African and Asiatic monkeys of the family Cercopithecidae. In this family, for which autopsies number 543, malignant tumors developed in 11 animals, but until the occurrence of the cases to be described no one species and only two genera were represented by more than a single case. Perhaps this is not entirely unexpected for until recent years the death rate was high, fertility was low, and the collection was made up of many species which were exhibited singly or in small groups.

One of the largest groups of Cercopithecus monkeys maintained at the Philadelphia Zoo is the rhesus macaque (*Macaca mulatta*). For these animals our autopsies number 95, but the only tumor-bearers found among them were the 4 cases presented here. In other institutions, however, rhesus monkeys occasionally have had neoplasms, although without involvement of the kidneys.[1, 2, 3] Thus, the development of renal carcinoma by 4 members of a small family group of these animals is entirely unparalleled in the known history of the species or, for that matter, of the larger group, the Cercopithecidae.

This family group consisted of the parents (2 females and a male) and 14 offspring (3 females and 11 males). Eight of these (1 female and 7 males) were from one mother (see Text-fig. 1). All members of the group now either are dead or were sold as excess animals long before their possible significance could have been

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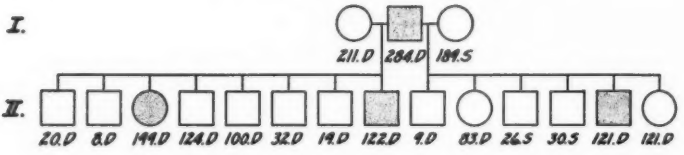
recognized, and as far as is known animals of the second generation did not reproduce. Family relationships, sex and exhibition periods of each of the group are given in Text-figure 1.

The parent animals were purchased as young adults, presumably captured in their native habitat. They and their offspring received routine care, which does not seem to have been entirely adequate since the average life span of the captive-born animals was much less than that of the parents — 80 months for one, 247 months for the other. Only 5 of the second generation lived to the minimal age of tumor development in this family — 121 months.



KEY:
■● CARCINOMA OF KIDNEY; □○ NO CARCINOMA.
NUMBER = MONTHS OF AGE AT END OF OBSERVATION; D = DIED; S = SOLD.

TEXT-FIG. 1. Pedigree chart for a family of rhesus monkeys, of which 4 members developed carcinoma of the kidney.

Their shorter exhibition periods apparently were due both to conflicts within the group and to an incomplete diet used during their growth period.

Four members of this family, the male parent and 3 offspring (2 males and 1 female), each developed a closely similar neoplasm of the left kidney. Descriptions of the cases follow the arrangement of the family chart, beginning with the male parent as the 1st case, the daughter as the 2nd case, her brother as the 3rd case and the half brother of these 2 as the 4th case (see Text-fig. 1).

The 1st animal lost weight for about 3 months before death and was killed because of its poor condition. At postmortem examination a spherical submucous leiomyoma partially obstructed the esophagus near its distal terminus, causing dilatation of the tube proximal to this point, and in the mucosa of the lesser curvature of the stomach a second tumor, a small sessile polyp, had developed. Mild atherosclerosis of the aorta and advanced cirrhosis of the liver were other incidental lesions. The cortex of the right

kidney contained several scattered, thin walled cysts measuring up to 1 cm. in diameter, but otherwise this organ was unchanged. Projecting from the lateral surface of the left kidney and covered by a thickened renal capsule was a soft, irregularly lobulated mass, 2 to 3 cm. in diameter, and mottled dark dull red and pale yellow. Within the organ a portion of cortex and medulla had been replaced by the spherical growth, which seemed sharply circumscribed except where a small part extended into the apex of the pyramid. The microscopic appearance of this and the other tumors to be described will be considered together.

The daughter lost weight for about 2 months before being killed as unfit for exhibition. Important pathological changes were limited to the renal parenchyma. The upper pole of the right kidney was occupied by a single thin-walled cyst, while in the left kidney more than half of the organ from the upper pole downward was replaced by a soft, pale yellow lobulated mass of tissue which expanded but did not penetrate the capsule.

The brother of this female received a severe injury to the back during a fight and was killed because of paralysis of the lower extremities. Except for atherosclerosis of the aorta and its larger branches, and localized inflammatory changes in the lumbar segment of the spinal cord, the left kidney alone attracted attention. The capsule of this organ had thickened and was adherent over a relatively large depressed part of the upper pole. On section through this area a firm, pale gray mass of tissue 2 to 3 cm. in diameter and roughly circular but indefinite in outline replaced a segment of both cortex and medulla.

The 4th animal of the series, half brother to the 2 preceding, died after a few days of indefinite illness. In his case a spherical thin walled cyst filled by semisolid, reddish brown material replaced the lower half of the left kidney. The outer surface of this cyst carried a reddish brown discoid nodule about 1 cm. in diameter, but other parts of this kidney were unchanged. The right kidney, however, was enlarged, firm, and studded with spherical pale gray masses of variable size. Lymph nodes of the retroperitoneal, the mediastinal, anterior cervical and tracheobronchial groups, as well as the surface of the diaphragm, also were involved by secondary growths.

The basic microscopic pattern of these tumors was closely simi-

lar, differences in histological appearance being due chiefly to hemorrhage and its effects upon the structure of certain of them. In each instance except the last the tumors were single isolated growths separated from the surrounding organ by a compact band of hyalinized connective tissue, apparently renal stroma, in which were remnants of kidney parenchyma. From this capsular structure fibrous tissue septums passed at random through the growths dividing them into irregular sections and joining the delicate stroma.

In each tumor epithelial cells were relatively large and pleomorphic, their cytoplasm more or less coarsely or finely vacuolated, and their nuclei correspondingly large and variable, sometimes gigantic and usually hyperchromatic. Mitoses were fairly frequent in tumors of the 1st and 4th animals, rare in the others. Multinucleated epithelial cells occurred in all, and in all, epithelium formed irregular cords, tubules and distorted acini. Often, too, relatively large spaces filled by papillary masses had developed. In all of these structures the epithelium presented bizarre arrangements, massing in multilayers and lacking polarity throughout (Figs. 2, 3 and 4). There were no definite differences in the microscopic appearances of any of the tumors that would permit any of the growths to be distinguished one from another.

Recent hemorrhages distended many spaces in tumors of the 1st and 4th animals. Associated with the well preserved erythrocytes were small collections of polymorphonuclear leukocytes and large numbers of pigment-bearing monocytes (Fig. 1). Certain parts of these growths had undergone necrosis and extensive accumulations of pigmented monocytes bordered such areas. Particularly was this true of the wall of the cyst which occupied the lower pole of the left kidney in the 4th animal. In this tissue an imperfectly circumscribed tumor mass extended from the outer to the inner parts of the cyst wall where pigment was most abundant. In its histological appearance this structure was identical with a part of the neoplasm in the 1st case, and entirely similar, except for greater size, to points of peripheral extension found in others of these growths.

Central portions of the tumors of the 2nd and 3rd animals had degenerated without hemorrhage having occurred, and in 1 of these other areas seemed partially atrophic, neoplastic cells being

smaller and more compact than elsewhere and the stroma increased in amount.

The histological make-up of the secondary tumors that developed in the 4th case was entirely similar to that of the other growths except that hemorrhage was not frequent and there was apparently rapid peripheral extension into normal tissue. In the kidney, for example, these metastatic growths surrounded many well preserved but isolated tubules and glomeruli.

Comment

Age appears to have been one factor in the development of tumors in this group of monkeys. This, of course, is true for many types of neoplasms of many species of animals and, in general, holds for captive wild mammals.[4] For other species of subhuman primates that have developed malignant growths while on exhibition in this garden, the average age has been more than twice as great as that for the whole group.

More important to the present cases than advanced age probably was heredity. The development of renal neoplasms in the male parent and the majority of offspring that lived as long or longer than the youngest tumor-bearer supports the assumption that he carried a dominant factor for this abnormality. It is unfortunate that the family may not be studied further but the time intervals necessary would have discouraged investigation.

Much more complete evidence of hereditary susceptibility to neoplastic disease is, of course, available for several species of laboratory animals.[5] The cases here reported show also that occasionally lower primates may supply more closely related material for comparison with instances of familial predisposition to tumor development in man.[6, 7, 8]

Specific classification of the tumors here reported is not an important consideration for the moment, nor does this seem an easy task. The gross appearance of a so-called hypernephroma was reproduced fairly completely in two tumors and partially in a third. Their microscopic make-up fulfils in part the sometimes accepted characteristics of hypernephroma as well as of adenoma or carcinoma of the kidney. Since such tumors of man are incompletely differentiated, nothing is to be gained by closer comparison. Whatever the origin of these neoplasms of monkeys,

their characteristics seem sufficiently similar to regard them as one type even though three were purely local growths.

That the left kidney was the primary site of development in the 4th animal of the series may be questioned, but the cyst found in this case may not be identified with any known developmental or degenerative formation. Rather it suggested a disintegrated tumor mass with a small growing remnant in its capsule.

Summary and Conclusion

Development of epithelial tumors in the kidneys of 4 members of a family of rhesus monkeys is described. Three of these neoplasms were local growths and the fourth metastasized widely, but the microscopic make-up of all was closely similar and seems to justify the diagnosis of carcinoma in each case. The relationship of these animals suggests that the factor of tumor development was inherited as a dominant trait which became apparent only after relatively advanced age.

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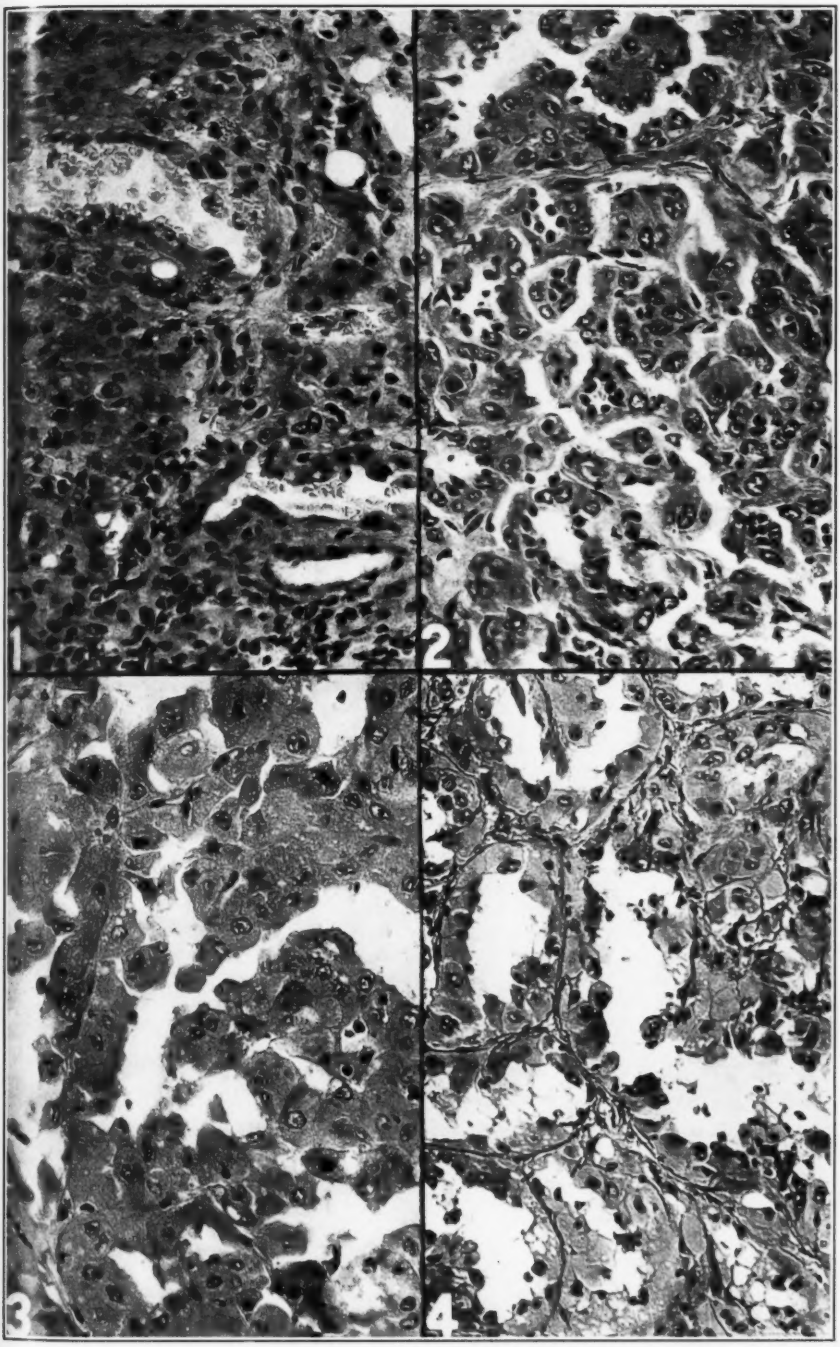
DESCRIPTION OF PLATE

PLATE 121

FIG. 1. An area of the tumor from the parent male in which hemorrhage occurred and pigmented monocytes and occasional polymorphonuclear leukocytes accumulated. Neoplastic cells are smaller than elsewhere in the growth. × 300.

FIGS. 2, 3, and 4. Fields from the tumors of the offspring, in the order in which they are described in the text, showing details of histological characteristics. × 300.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TERATOMA OF THE OVARY, WITH A SEGMENT OF VERTEBRAL COLUMN AND REMNANTS OF NOTOCHORDAL TISSUE *

Report of a Case

Joseph Luc Riopelle, M.D.

(From the Institute of Pathology, University of Montreal, and the Pathological Laboratory, Hotel-Dieu, Montreal, Canada)

The genesis of teratomas is still one of the most controversial questions in pathology. One of the main reasons for this controversy is that up to the present time many of their constituents have not been properly identified. Among the fundamental structures, the existence of which is most contested, least established are inclusions of notochordal tissue and segments of vertebral column, according to authorities such as Nicholson [1] and Willis.[2] The latter writes in a recent paper: "I have searched diligently for notochordal tissue in my specimens, but without success, and I am more than sceptical of the few recorded examples of alleged notochordal tissue in teratomas. Of the rare specimens of supposed 'vertebral columns' in teratomas, not one has been adequately studied."

It may be conceded that in the few observations already published the histological description is at times sketchy. Moreover, except in rare instances, the axial skeleton is, as a rule, very rudimentary. As for chordal remnants, I do not know to what instances Willis refers. The only observation mentioned by Miller [3] is that of Bertone, which I have not been able to consult.

I recently had the opportunity to study a teratoma of the ovary containing remnants of notochordal tissue, a segment of vertebral column, and a rudiment of neuraxial tissue. The interest of the following observation lies more in its theoretical implications than in its rareness.

REPORT OF A CASE

Clinical History: Miss S. R., 27 years old and unmarried, was admitted Dec. 22, 1938, to the Hotel-Dieu, complaining of severe abdominal pain. On

* Received for publication February 5, 1940.

the evening of admission, while reaching for a hat box on a high shelf, she overstretched and bent backwards, and was seized with a sudden violent pain in the lower abdomen, accompanied by nausea. The pain had persisted, despite opiates, since its onset.

On physical examination the patient was found to be in good general condition but in obvious pain. On palpation of the hypogastrium a hard, tender, well defined mass was felt. A diagnosis of ovarian cyst with a twisted pedicle was made and an operation performed the same day.

On opening the pelvic cavity considerable blood was found. The left adnexa formed a reddish purple mass about 16 by 10 cm., twice rotated on its pedicle. On the right side was another ovarian cyst about 10 cm. in diameter.

A left salpingo-oophorectomy was performed. Attempted dissection of the right ovarian cyst in order to save a portion of the ovary was unsuccessful as the wall of the cyst was only a thin shell enclosing sebaceous matter and hair. The right adnexa and the appendix were then removed.

The postoperative course was uneventful and the patient was discharged 8 days after the operation.

Gross Examination

The specimen removed from the left side was seen by the pathologist after gross fixation in saline-formalin. It weighed 410 gm. and consisted of a swollen, dark red fallopian tube, connected by a short mesosalpinx to a large infarcted ovary about 16 by 10 cm. (dimensions after fixation about 9 cm.).

On the side opposite the mesosalpinx was a linear incision along the main axis of the specimen. This incision, made by the surgeon in the operating room, opened the cavity of a single cyst, through a hardened wall, 5 to 10 mm. in thickness. Sebaceous matter and hair protruded through the half opened gap. A complete section of the ovary was then made through the preceding incision.

The cut section revealed a completely infarcted ovarian stroma, also the mesosalpinx and tube. The cyst contained, in addition to a small quantity of sebaceous matter, a pedunculated mass arising in the vicinity of the hilus of the ovary.

The internal surface of the cyst was smooth, free from hair, and apparently lacking an epithelial lining. The projecting mass, on the contrary, showed a grayish, wrinkled, skin-like surface, bristling with short hair, except on one side where a tuft of gray hair, about 10 to 12 cm. in length, covered an area about 2 cm. in diameter. On the side opposite to the scalp-like area two sessile symmetrical projections, bent contrariwise to the

point of implantation of the dermoid and flattened on its surface, were seen. There was no trace of nails at the tips of these limb-like formations.

The specimen was cut according to the plane of symmetry of the dermoid, that is, through the pedicle, the tuft of hair and the midline between the "limbs," except that the incision barely touched one of them. In addition to the dermoid, the teratoma was seen to be formed of an extracystic portion, included in the ovarian stroma and attached to the dermoid by means of the pedicle so that the tumor had the shape of an hour-glass.

The major part of the dermoid was formed of fat divided in lobules by thin fibrous septums. This fat contained small hemorrhagic foci, more numerous and in places confluent in the region of the pedicle. In the center lay an elongated osseous formation, measuring 15 mm. and consisting of three cubical segments of bone filled with red marrow, placed in linear series, and separated by a white glistening substance similar to cartilage. Its orientation was oblique in regard to the pedicle. The gross examination suggested a segment of vertebral column.

Histological Examination

Due to torsion of the adnexa, the whole extracystic portion of the teratoma had undergone severe hemorrhagic infarction, so that the tissues were badly damaged, although still recognizable. The dermoid, on the contrary, was not infarcted, and showed but few hemorrhagic foci. However, the most delicate tissues were somewhat altered, either by anoxemia or by delayed fixation of the deeper portions of the mass. In any event, the segment of vertebral column, the neuraxial tissue and the remnants of the chorda, *e.g.* the most interesting features of the teratoma, were still preserved. Let us begin with the study of the structure just mentioned.

Segment of Vertebral Column with Neuraxial Tissue

The section of the dermoid according to its plane of bilateral symmetry also cut the axial skeleton and neuraxis in two symmetrical parts. Each half thus obtained was embedded in paraffin after decalcification. Unhappily, the faulty decalcifica-

tion of the first block made sectioning difficult. However, random sections, at various levels, were obtained. The other block was sectioned and studied in series (400 sections at 1/150 mm.). The following description is a summary of the total information gathered.

The axial skeleton measures 15 mm. in length, 5 to 8 mm. in thickness, and, as far as can be ascertained by serial sections, about 6 mm. in width at the superior extremity. Its shape is that of a cylinder, slightly flattened anteroposteriorly, opened at both ends, and funnel shaped at the top. On the middle line the structure of the vertebral body is almost entirely cartilaginous. Bilaterally the cartilaginous primordium is for the greater part replaced by three centers of ossification in a linear series. The central part of these formations consists of a very cellular marrow; the periphery at both ends is formed of a thin core of bone, almost reduced to the zone of the erosion line, because the osseous tissue seems to disappear by osteolysis as soon as it is formed. On all sides, on the contrary, the bony shell is thicker and more consistent and covered with a fibrous membrane (periosteum).

On each side are three of these ossification centers, the lower one noticeably smaller and curved backwards. The symmetry of these formations does not seem perfect. In one of the two halves the center one is very large, and shaped like an hour-glass, as though formed by confluence of two independent centers.

Laterally the vertebral bodies are continuous with flattened bony blades, which are curved backwards and represent posterior arches. At their junction with the body of the vertebral column these arches are fused into a single mass, bored with round holes through which run myelinated nerves. The canals, therefore, are analogous to the foramina intervertebralia. They number three on the left side and four on the right. There is no trace of differentiation of articulary apophysis, or of articulations.

Only the two superior neural arches are completely individualized and joined at the middle line. The lower one is incomplete and leaves a large gap on the posterior surface. The neural arches are formed of a thick shell of bone richer in calcium salts than that of the vertebral body. Their shafts are filled with active hemopoietic marrow. Near the superior orifice of the vertebral rudiment, and slightly encroaching on it, there is on the left a

block of compact bone which does not seem to be connected with it.

The "vertebral canal" is occupied by a cylindrical mass of neuroglia interspersed with nerve cells. Its inferior extremity tapers into a conic projection which protrudes through the inferior opening of the canal and is likewise slightly curved backwards. At the superior orifice it is continuous with a round mass of central nervous tissue enclosed in a thin fibrous membrane. The brain-like formation is developed on the right side only.

On each side the neuraxial tissue is flanked by a series of ganglia. Each of these ganglia shows typical nerve cells. They are connected on one side with the neuraxial cylinder, and on the other, send forth a large myelinic trunk which enters the corresponding foramen intervertebrale. There are three ganglia on the left side and four on the right, but the superior one of the four is in close contact with the brain (though wedged in the vertebral canal), so that it is in all probability related to it.

Remnants of Chorda Dorsalis

In the rudiment of vertebral column, but nowhere else, are found islands of a vacuolar tissue having the typical aspect of chorda dorsalis remnants. Such a diagnosis would seem to be correct considering Figures 3, 4, 5 and 6. These show well circumscribed masses formed by groups of vacuolar cells, each of which is enclosed in a sheath of fibrillary collagen. At high magnification the identity with physalipherous cells is still more remarkable. One sees collections of cavities, either small or very large, separated by thin cytoplasmic lamellae, with oval, finely woven nuclei at the points of junction of the cytoplasmic septums. The contents of the cavities are colorless. Unfortunately no tissue was available for mucus or glycogen stains.

These masses were only found in the right half of the rudimentary spine, and that, laterally, about midway between both extremities. One of these masses is set in a bony notch and covered over by the periosteum. The others are enclosed in the vertebral body. One is elongated transversely to the length of the axial skeleton, and extends from the anterior to the posterior face of the body of the vertebra.

The dimensions of these masses are large, and vary from 1 to

2 mm. They are quite gigantic in comparison with the size of the "vertebrae," and are even larger than the notochordal strands in embryogenesis. Another remarkable peculiarity is the variability and the large size of the physalipherous cells.

Other Tissues of the Teratoma

It will suffice to mention the cutaneous lining of the dermoid, with its numerous pilosebaceous adnexa and sweat glands. This lining covers the whole of the dermoid, together with its pedicle. The wall of the cyst itself seems devoid of any epithelial lining. This has been observed in other fetal teratomas where the cavity of the cyst has been compared to an amniotic sac.

Here and there in the fat are cartilaginous nodes and striated muscle, remarkably rich in typical neuromuscular spindles. One may also observe numerous tactile corpuscles, most of which are paciniform or atypical.

In the immediate neighborhood of the anterior aspect of the "spine" is a chain of unmyelinated fibers and sympathetic ganglia with large islands of paraganglion cells. In front of these formations are ducts with a narrow lumen and a simple cubic epithelium, resting on a thick muscular sheath and recalling remnants of wolffian canal. In their vicinity, but without continuity with them, are two well limited masses consisting of intercommunicating tubules. Unhappily the epithelium of the tubules is exceedingly altered and transformed into a shapeless magma. However, a few remnants show a pluristratified cylindrical ciliated epithelium, suggesting efferent ducts, but the condition of the tissue does not permit a positive identification.

Among the organs that have suffered most from delayed fixation is a duct with a malpighian mucosa and lined with a thick muscular coat. That duct (esophagus?) extends through the pedicle of the dermoid and down to the extracystic portion of the teratoma.

The extracystic portion is enclosed in a fibrous capsule. It is formed almost entirely by a loop of intestine, with typical crypts of Lieberkühn. There is, moreover, a crescent-like empty cavity with a flattened lining very similar to the peritoneal endothelium.

The surface of implantation of the extracystic portion of the teratoma shows nothing suggesting placenta.

Comment

In the above described case the identification of a rudimentary vertebral column with a spinal cord is established by the following data: (1) localization in the plane of symmetry of the teratoma; (2) orientation according to that plane of symmetry which cuts the bony formation in two halves; (3) general conformation of the axial skeleton, which is shaped like a flattened cylinder with a central "vertebral canal"; (4) bilateral centers of ossification in linear series, giving the cartilaginous primordium a segmented aspect; (5) the presence of a neuraxis, bilaterally flanked by spinal ganglions; and (6) inclusion of large remnants of typical notochordal tissue.

The peculiarities of the vertebral segment call for the following comment:

Asynchronism and Independence of Ossification in the Vertebral Body Versus that of the Neural Arches: The latter are completely ossified, while the former shows incomplete ossification followed by partial resorption. The independence of both processes of ossification, the precession of that of the neural arches are well known facts (Junghanns [4]). In the case reported here the formation of posterior arches and their ossification must be related to the presence of a neuraxis for, as we know, the latter influences the genesis of the posterior arches, as demonstrated by malformations. On the contrary, the incomplete ossification and the osteolytic process occurring in the body of the vertebrae are more obscure. Perhaps what is known about the mechanical factors in bone formation must be taken into account.

Abnormalities of the Remnants of Chorda Dorsalis: These remnants are situated posteriorly and laterally in the vertebral body. Schmorl [5] has shown that such remains may be found in any part of the body of the vertebra. Worthy of note are their large size, not only in proportion to the vertebral body, but also in absolute dimensions. This fact, coupled with the irregular size of the cell, suggests the possibility of a tumor proliferation.

Discussion and Conclusions

In a previously quoted paper Willis,[2] in accord with Nicholson,[1] rejects all current hypotheses on the origin of teratomas, particularly the twin inclusion and parthenogenetic theories.

Although recognizing that some tissue correlations analogous to that of ontogeny are observed in these tumors, he stresses the absence of true organs and somatic regions, the excess of some components and absence of others, and the abnormal mixture of tissues. But his most important reason is that "with extremely rare and doubtful exceptions, teratomas exhibit no signs whatever of axiation, metameric segmentation, or delamination of germinal layers, *i.e.* the earliest and most fundamental features of a soma are lacking."

It is true that few teratomas seem to possess the above mentioned characteristics (Meyer [6] and Kaboth [7]). At all events the case reported here is far from a doubtful exception. Here, it is evident that an orderly development has taken place, homologous to that of a soma. Although all major tissues are represented in the tumor, it is conceded that its growth has been for a great part abortive. That failure to develop is not surprising, unless we unduly assume all conditions of growth to be the same in a fetus and in a teratoma, in particular as regards circulation. That subsequent modifications may tend to obliterate the signs of organization is also shown in the specimen. In the body of the spinal column the newly formed bone is resorbed, while there is a tendency for ossification centers to fuse together. Had the tumor been removed belatedly, it is probable that the evidence of metameric segmentation would have been partly destroyed. Hence, we cannot ascribe an absolute value to the ideas of Willis and Nicholson, although they have contributed much to our knowledge of teratomas.

NOTE: I am deeply indebted to Dr. Louis C. Simard for the photographs and to Dr. G. Manseau for the drawing of Figure 1.

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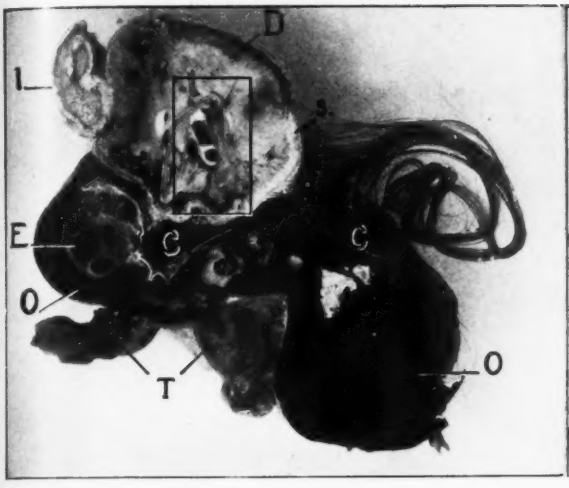
DESCRIPTION OF PLATES

PLATE 122

FIG. 1. Whole section of the left adnexa uteri, cutting the teratoma according to its plane of symmetry. T and O = fallopian tube and ovary, swollen by hemorrhagic infarction; C = cystic cavity (previously opened); D = intracystic eminence covered with skin; S = "scalp"; I = one of the "limbs"; and E = extracystic portion of the teratoma (chiefly intestinal loops).

FIG. 1a. Drawing of the inset in Fig. 1. VC = body of the vertebral column; NA = neural arches; B = brain-like projection of nervous tissue; and SC = spinal cord.

FIG. 2. Sagittal section of the vertebral segment and neuraxial tissue near the middle line. VC = cartilaginous primordium of the body of the vertebral column; NA = neural arches; SC = spinal cord made of neuroglia interspersed with nerve cells; E = remnants of ependyma; and SG = spinal ganglion.



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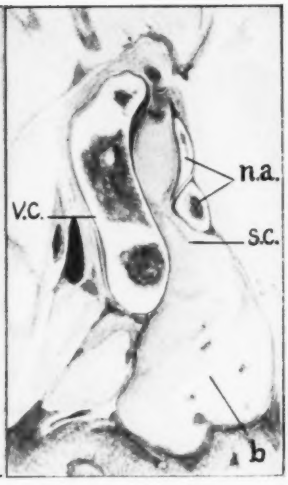

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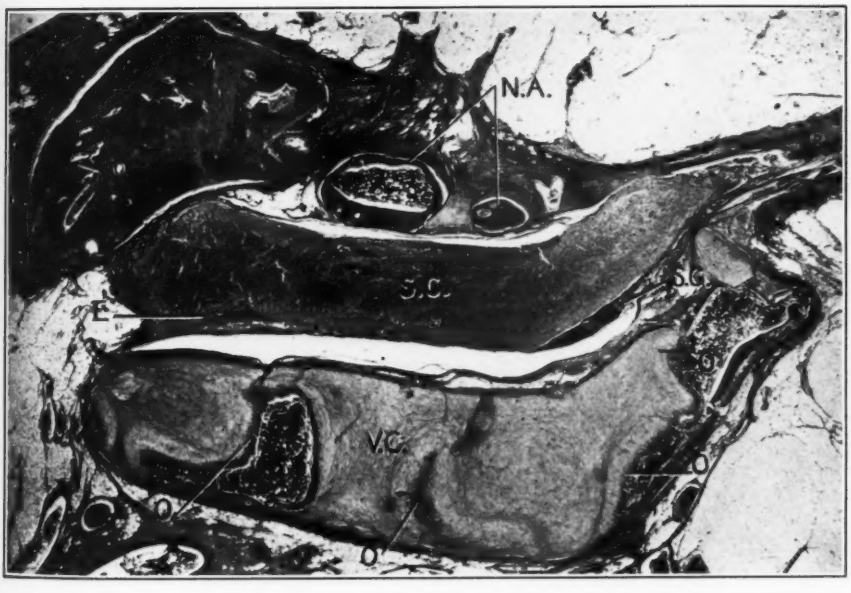

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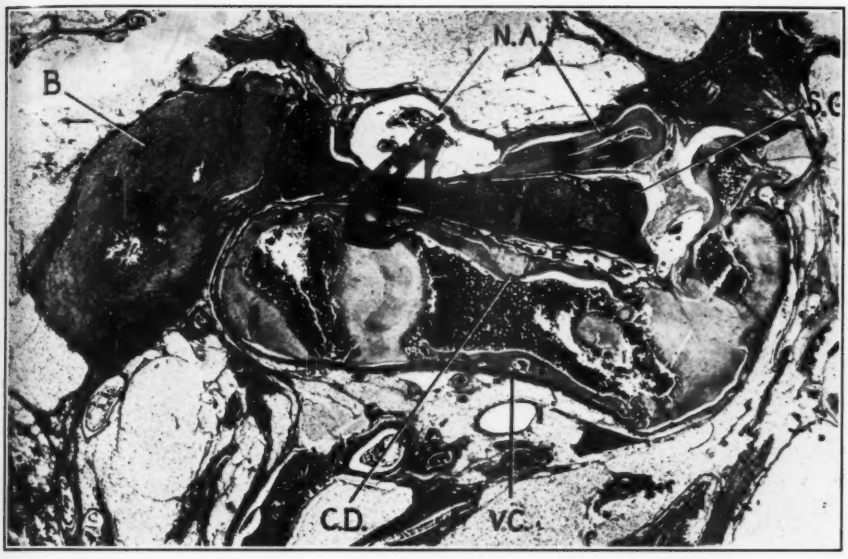
PLATE 123

FIG. 3. Sagittal section, lateral region, right side. VC = body of the vertebral column; O = ossification centers; NA = neural arches; B = "brain" (present on one side only); SG = chain of spinal ganglions. Dotted regions are filled with nerve cells; compact regions are bundles of nervous fibers; CD = island of chorda dorsalis.

FIG. 4. Same side. Section running almost tangentially to the side of the spine. VC = body of the vertebral column; NA = neural arches fused with the vertebral body; N = transverse sections of nerves running through the foramina intervertebralia; CD = islands of chorda dorsalis; and B = "brain" tangentially sectioned.



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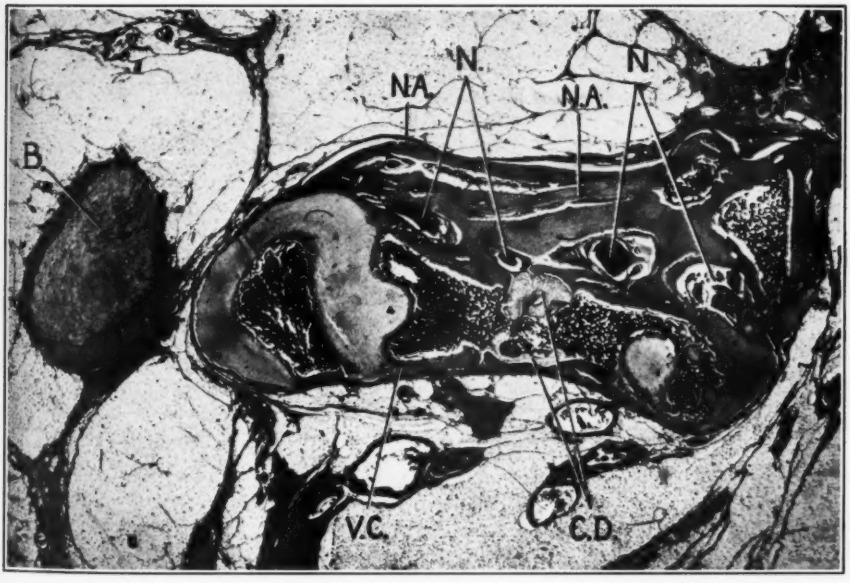

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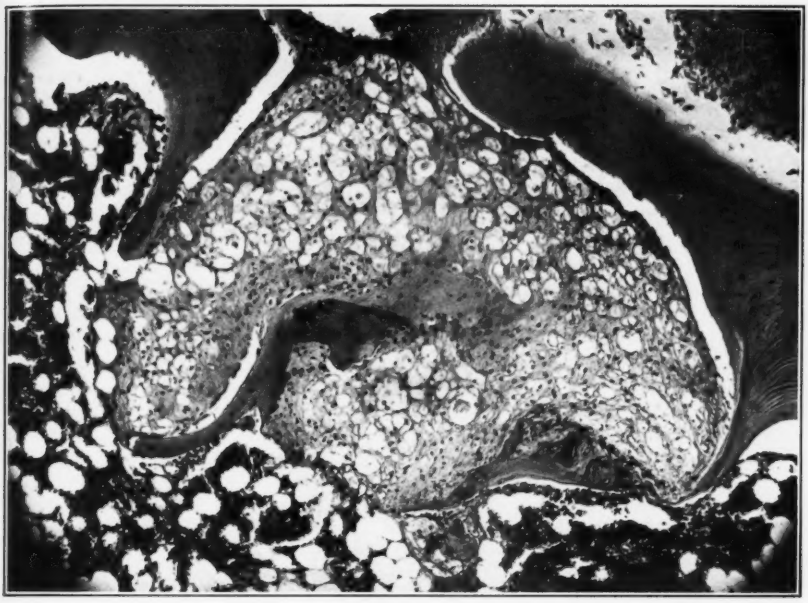
PLATE 124

FIG. 5. Island of chorda dorsalis; same section as in Fig. 4. Below, hemopoietic bone marrow.

FIG. 6. High magnification of notochordal tissue taken from the island in Fig. 5.



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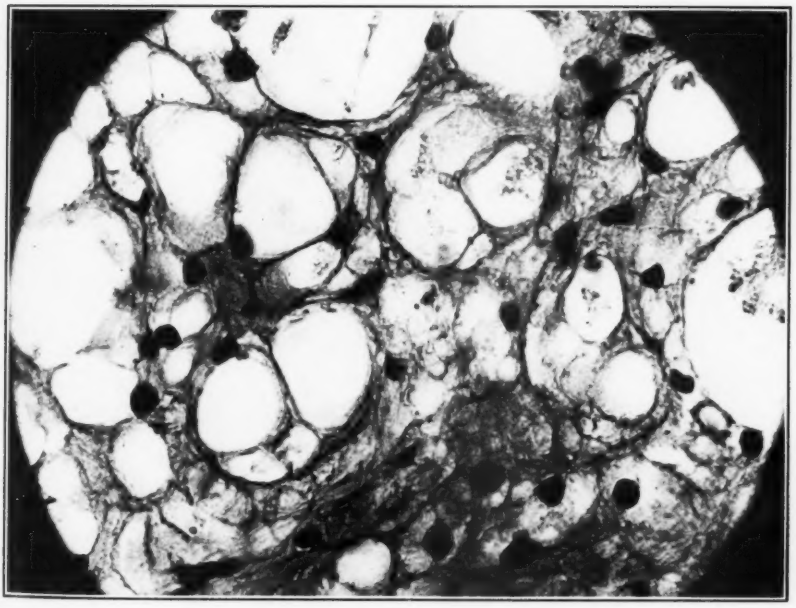

6

Riopelle Teratoma of the Ovary

SCIENTIFIC PROCEEDINGS OF THE FORTIETH ANNUAL MEETING

OF THE

AMERICAN ASSOCIATION OF PATHOLOGISTS AND BACTERIOLOGISTS

HELD IN PITTSBURGH, PENNSYLVANIA

MARCH 21ST AND 22ND, 1940

BUSINESS MEETING

OF

THE AMERICAN ASSOCIATION OF PATHOLOGISTS AND BACTERIOLOGISTS

Held in the Mellon Institute for Industrial Research, Pittsburgh, Pennsylvania

March 21, 1940

PRESIDENT WELLER IN THE CHAIR

The Secretary announced the resignation of Dr. Frank B. Mallory as Treasurer of the Association and Editor of the American Journal of Pathology.

The Secretary moved the adoption of the document which follows and this was seconded in a gracious address by Dr. Stanhope Bayne-Jones. The statement was adopted unanimously.

FRANK BURR MALLORY was one of the original group which established the American Association of Pathologists and Bacteriologists. He was present at the first annual meeting in Boston, April 5 and 6, 1901, and on Saturday, April 6, 1901 contributed to the first program with the presentation of a paper entitled, "On Liver Necroses." He was elected a member of the Council in 1905 to begin his service in 1906. In 1910, at the Washington meeting, he served as President of the Association. At the Philadelphia meeting in 1912, he was elected Treasurer, and has served in that capacity continuously for 28 years. In 1924 he was elected Editor-in-Chief of the new American Journal of Pathology, the first issue of which appeared in January, 1925, and has served continuously as Editor-in-Chief since that time.

The Council now reports with deep regret the resignation of Doctor Mallory from both the positions which for so long a time he has filled with distinction. His service to the Association is not measured by the bare recital given above. His advice in the conduct of the business of the Council has been wise and sound. His attention to the positions he has held has been noteworthy for his intense devotion to the highest ideals and his meticulous care as to details. He has so administered the treasurer's office as to insure the financial stability of the Association. His editorship has placed the American Journal of

Pathology in the front rank of scientific periodicals. His attendance at meetings has been faithful throughout these many years, and thereby his scientific spirit and integrity have operated as a significant stimulus to his fellow members and to pathology as a field of profitable study. The position of respect which has been gained by the Association as a scientific body is in large measure due to the high standards, the attainments, the loyalty, the enthusiasm and the great personality of Frank Burr Mallory.

On recommendation of the Council, it is voted to place this note of appreciation of the man and his service to the Association and Medicine on the minutes of this meeting, to order it published in the Proceedings of this meeting, and to extend to Doctor Mallory congratulations upon his distinguished position in Pathology and best wishes for a happy future.

On nomination of the Council the following officers were elected by the Association:

President	STANHOPE BAYNE-JONES
Vice-President	SAMUEL R. HAYTHORN
Secretary	HOWARD T. KARSNER
Treasurer	ALAN R. MORITZ
Incoming Member of Council	MALCOLM H. SOULE
Assistant Secretary	FRANCIS BAYLESS
Assistant Treasurer	GRANVILLE A. BENNETT

The Secretary announced the election of the following new members:

Max Appel	Saul Jarcho
Llewellyn L. Ashburn	Erik Johannes Kraus
Guy Darrell Ayer, Jr.	Stuart Wellington Lippincott
Clemens Ernst Benda	Warren Bond Matthews
Kenneth M. Brinkhous	David Raymond Meranze
Henry Brody	Robert F. Norris
Milton J. Eisen	John Musser Pearce
George H. Fetterman	Thomas Martin Peery
Robert Fienberg	Hans P. Popper
Edward A. Gall	Joseph Edward Porter
Hugh G. Grady	Margaret G. Smith
William E. B. Hall	Aaron Hirsh Stock
Albert H. Harris, 2nd	Herbert F. Traut
George Hartley, Jr.	Harold Wood

The Secretary drew attention of the Association to the fact that the constitution provides for the transaction of the business of the Association by the Council. He then announced the election of

Dr. Carl V. Weller as Editor-in-Chief of the American Journal of Pathology. He stated that Doctor Weller had agreed to serve in this capacity for a term of one year, during which he could decide whether or not his many other duties and commitments would permit him to undertake the task for a longer period.

The Secretary announced the election of Dr. Tracy B. Mallory as Assistant Editor, stating that the Council had decided that the office of Assistant Editor should be for a term of one year.

The Secretary announced that a Board of Editors, composed of six members, would be selected but that the Council will not act upon this matter until further thought is given it by Doctor Weller and Doctor Mallory. Each member of the Editorial Board will be elected for a period of six years, so adjusted that one appointment expires each year.

Voted to accept with regret the resignations of Drs. T. H. Belt, M. M. Canavan, R. I. Cole, E. V. Cowdry, W. H. Manwaring, Joseph McFarland, J. L. O'Leary, Oscar Richardson, G. H. Scott, R. E. Shope, Richard Thompson and Margaret Warwick.

Voted to record with deep regret the deaths of Drs. Maurice Brodie, D. M. Cowie, B. L. Crawford, Harvey Cushing, F. P. Gay, W. H. Park, L. H. Prince, Alfred Stengel, P. G. Weston and H. U. Williams.

The Secretary announced that the next meeting will be held as the guests of New York University in New York, April 10 and 11, 1941.

The Secretary announced that it had been voted to have a symposium on "Shock" and to appoint Dr. Virgil H. Moon as referee.

The scientific program follows:

AMERICAN ASSOCIATION OF PATHOLOGISTS AND BACTERIOLOGISTS

SOLID OVARIAN TUMORS. Malcolm B. Dockerty (by invitation) and William Carpenter MacCarty, Sr., Rochester, Minn.

Abstract. Our particular purpose on this occasion is to present a practical review of a large series of ovarian solid tumors, personally studied, so that teachers and students may be able to comprehend more readily what has been a rather incomprehensible subject. One of us (Dockerty) has studied more than 500 solid ovarian tumors from which series he has already reported 30 granulosa cell tumors (the feminizing type), 4 arrhenoblastomas (the masculinizing type), 9 dysgerminomas (the neutralizing type), 10 theca cell tumors, 10 Brenner tumors and 131 fibromas. One of our assistants, Dr. Leffel, has for publication a series of 44 Krukenberg tumors. The rest of the series, composed of sarcomas, adenocarcinomas and solid teratomas, is in the final stages of preparation for publication.

The accompanying tables are self explanatory and serve merely as a brief outline of the essential general differences between the various groups. Very frequently things seeming to be entities in a small experience are found to be closely related in a large experience. Ovarian solid, and indeed cystic, tumors are no exception to this rule; they present many characteristics in common so that in all probability the different tumors have their origin in the same developmental structures with varied potentialities. Only the granulosa cell tumor, the arrhenoblastoma, the dysgerminoma and the Krukenberg tumor seem to have clinical manifestations which in some cases present criteria for a fairly accurate preexploratory diagnosis.

Discussion

(Dr. Walter Schiller, Chicago, Ill.) I want to congratulate Dr. Dockerty and Dr. MacCarty for their excellent presentation and for their charts which give a very clear picture of the pathological and clinical characteristics of the tumors.

I should like to mention that in the last few years I have had the opportunity of examining a number of solid ovarian tumors which represented mixtures of various types. The combination of granulosa cell tumor and dysgerminoma is well known, and so is the combination of granulosa cell tumor and arrhenoblastoma. Recently I have seen combinations of hypernephroma and dysgerminoma, as well as of hypernephroma and teratoma. Sometimes we have great difficulty in classifying ovarian tumors when the slide we examine gives no explanation for some hormonic activity observed clinically with the tumor. When we examine slides of different parts of such a tumor we may find out that there is a mixture of different types and the tissue which was not represented by the first slide is responsible for the hormone activity.

To get the correct classification and interpretation of solid ovarian tumors I feel it of great importance to cut the gross specimen into thin slices, to fix these properly, and to get at least one block of every slice — if possible, several blocks. This is the only way to recognize mixed tumors as such.

TABLE I

Comparative Data on Solid Ovarian Tumors (Dockerty)

	Primary adenocarcinoma	Sarcoma	Teratoma	Krukenberg tumor
Histogenesis	Probably begins as cystic tumor (cystadenoma)	60% from fibromas; 40% from stroma of ovary	Mutation of cells or rests (?)	Always metastatic: stomach (80%), jejunum, colon, gall-bladder, breast, hepatic duct, uterus
Age	Any age	Any age, usually earlier than carcinoma	Any age	Usually less than 45 years; average age 48.2 years
Characteristic symptoms or syndrome	Rapidly growing tumor; cachexia; frequently ascites	Non-specific	Non-specific	Often gastric
Unilateral or bilateral	50% bilateral	20% bilateral	15% bilateral	35% bilateral
Gross appearance	Solid, brown, often adherent; often necrotic and cystic	Not diagnostic	Variety of tissues found	Solid smooth surface; cut surface becomes concave
Microscopic pattern	Malignant cells with tendency to formation of glandular spaces	Usually fibrosarcoma	Heterogenicity of tissues	Often signet-ring cells
Prognosis	Malignant	Malignant	Sometimes malignant	Malignant
Hormone elaborated	No hormones	No hormones	?	No hormones

	Theca cell tumor	Fibroma	Brenner tumor
Histogenesis	Theca of follicle	Ovarian stroma	Embryonic rests (?); teratomatous origin (?); mutation of cells
Age	80% after menopause	100% after puberty	70% after menopause
Characteristic symptoms or syndrome	Often periodic postmenopausal bleeding (as in granulosa cell tumor)	Slowly growing tumor; ascites in 10% with large tumors; occasionally also hydrothorax *	Symptoms not characteristic
Unilateral or bilateral	100% unilateral	90% unilateral	95% unilateral
Gross appearance	Solid, yellow, fibrous; uterus large	90% solid, white	70% solid, resembling fibroma; 30% solid nodules in walls of pseudomucinous cysts
Microscopic pattern	Fibroblastic cells laden with fat; hyaline bands	Cellular and fibrous types; small fibroblastic cells	80% fibrous stroma; 20% squamous cells in islands with cystic centers; mucus
Prognosis	Sometimes malignant	Benign (if not fibrosarcoma)	Benign
Hormone elaborated	Estrin	No hormones	No hormones

* Meigs' syndrome

TABLE II

	Granulosa cell tumor	Arrhenoblastoma	Dysgerminoma
Histogenesis	Granulosa cells	Testicular "rests"	Undifferentiated sexual cells
Age	40–60 years (60%)	20–40 years (90%)	5–20 years (70%)
Characteristic symptoms or syndrome	Precocious menstruation, early sex development, postclimacteric menstruation, feminization	Sterility, hirsutism, atrophy of breasts, hypertrophy of clitoris, voice changes, amenorrhea, masculinization	Pseudohermaphrodism; can have normal menstruation and pregnancy; hypoplasia of genital organs
Unilateral or bilateral	95% unilateral	100% unilateral	65% unilateral
Gross appearance	Solid, granular, homogeneous	Solid, multicentric nodules	Solid, consistence of brain
Microscopic pattern	Folliculoid, cylindroid, diffuse	Tubular, cylindroid and diffuse with interstitial cells	Undifferentiated cells, giant cells, lymphocytes
Prognosis	Rarely malignant (5%)	Frequently malignant (25%)	Very frequently malignant (45%)
Hormone elaborated	Estrin	Male sex hormone	No hormones

(Dr. Walter M. Simpson, Dayton, Ohio.) After conversing with Dr. Virgil Counsellor at the Mayo Clinic a few years ago, and after reading the publications of Drs. MacCarty, Dockerty, Broders and Desjardins regarding ovarian tumors, we adopted this classification. We have found it very useful.

(Dr. MacCarty, closing.) I want to express my appreciation to Dr. Schiller. No one who studies ovarian tumors can fail to know of the work of Dr. Schiller and also of Dr. Robert Meyer. We are fortunate to have these two gentlemen in the United States. Dr. Schiller, as most of you know, is in Chicago, and Dr. Meyer is in Minneapolis, and I feel very proud that we have them here with us in this country, because I must confess that I think we know more about solid ovarian tumors in this country than in any other place in the world. I must add to that I do not mean myself, but my associate, Dr. Dockerty. I think these three (Dr. Schiller, Dr. Meyer and Dr. Dockerty) know more about this subject than anyone else.

MIXED MESODERMAL TUMORS OF THE BODY OF THE UTERUS. Averill A. Liebow and Robert Tennant, New Haven, Conn.

Abstract. Mixed tumors may occur anywhere in the genitourinary system. In the body of the uterus about 1 of every 7500 malignant neoplasms contains heterologous mesodermal elements. Three cases that came under personal observation are reviewed, together with the records of 59 cases described by others.

Clinically the tumors affected women beyond the menopause (average age 56.4 years). They manifested themselves almost always by sanguineous vaginal discharge, lacking only in 6 of 47 individuals. Pain was more prominent in the anamnesis than with carcinoma of the uterus. The average duration of symptoms was 29 weeks. Only 4 of 29 patients were known to have survived 2 years or more. Nineteen of 28 were known to have succumbed. Aside from immediate postoperative deaths, these patients died usually about 33 weeks after the diagnosis was made, and 52 weeks after onset of symptoms, despite the radical hysterectomies that were performed in most instances.

The tumors are usually polypoid and take origin from the posterior wall of the cornu, but may obliterate the endometrial cavity or take the form of multiple papillae, replacing the endometrium. They consist of an undifferentiated stroma embedding islands of cartilage, striated muscle, and sometimes both, mixed often with epithelium or other tissues such as smooth muscle or nervous elements. Local recurrences occurred in all 16 patients known to have died of metastases, but in 4 or 5 of those that were autopsied metastases were found also in the lungs and the pleura. The histology of the metastatic lesions in some cases is identical with that in the primary growth, but in others only some of the many varieties of tissue are seen, and in some only the dedifferentiated sarcomatous stroma is found.

Evidence concerning the histogenesis gathered from the 3 specimens studied supports Wilm's concept of the intra-Müllerian inclusion of multipotential germs, possibly from segmented embryonic mesoderm, rather than the theory of metaplasia. Reticulum stains demonstrated that the argyrophil fibers dispose themselves about the elements of the tissue as though they were respectively true cartilage, epithelium and sarcoma, and when a portion of cartilage-like tissue from 1 specimen was cultured *in vitro* the explant behaved in a manner analogous with that from fetal cartilage.

Discussion

(Dr. Warren C. Corwin, New Brunswick, N. J.) I should like to ask Dr. Liebow about metastases in these tumors.

(Dr. Liebow.) It is generally stated that metastases occur locally, and that only rarely are distant metastases to be found. However, in 4 of the 5 cases in which autopsies were performed metastases were found in the lungs. In our first patient the operation was performed with the full knowledge that the roentgenographic examination showed metastases to the lungs. The patient died 6 months following the operation. The second patient had local recurrences with intestinal obstruction 11 months after the operation, and died 13 months after it.

(Dr. Walter Schiller, Chicago, Ill.) The tumors described by Dr. Liebow evidently are not too rare. I remember having seen 1 in which the pathologist who saw it first made the diagnosis of incomplete abortion, based on the presence of immature cartilage and immature embryonic epithelium in the slide of the curetting. Later on, with careful examination of the history and the slide, it was found that there was no abortion but instead a mixed teratoid tumor of the uterus was present. Inclusions of cartilage in the uterine wall have been found and have been analyzed in an excellent paper by Dr. Robert Meyer. Whenever in a curetting immature cartilage is found, or for that matter any embryonic tissue, the differential diagnosis between abortion and mixed tumor of the uterine wall should be considered carefully.

(Dr. Edwin F. Hirsch, Chicago, Ill.) I should like to ask Dr. Liebow as to the origin of the cartilage — whether he thinks it arises in the connective tissue multicentrically or in one place.

(Dr. Liebow, closing.) We believe that Wilms' original explanation, or some modification, is probably correct; namely that these tumors represent inclusions within the Müllerian duct system, or some undifferentiated tissue, probably dorsal mesoderm, early in embryonic life, and that subsequently this may develop into the various forms to which the mesoderm may give rise.

GLIOMAS OF MIXED TYPE. Jesse E. Edwards and William O. Russell (by invitation), Boston, Mass.

Abstract. A series of 47 cases of cerebral and cerebellar gliomas was studied in order to learn the incidence of mixed types in specimens taken at one time. The number of blocks of tissue taken varied from 1 to 10 per case, the mean being 3.

In 39 of the 47 cases one type of glioma was seen, while in 8, or 17 per cent of the cases, a mixture of several types of glioma was observed. In any one tumor the cell types represented were not always closely related developmentally. In 2 instances astrocytoma and glioblastoma multiforme were present in the same tumor, and in 1 instance areas of oligodendroglioma were mixed with those of glioblastoma multiforme.

The types observed in the 8 cases of mixed type of glioma are as follows:

1. Ependymoma, glioblastoma multiforme.
2. Astroblastoma, glioblastoma multiforme.
3. Astrocytoma, glioblastoma multiforme.
4. Polar spongioblastoma, glioblastoma multiforme.
5. Ependymoma, glioblastoma multiforme.
6. Medulloblastoma, polar spongioblastoma, glioblastoma multiforme.

7. Astrocytoma, polar spongioblastoma, glioblastoma multiforme.
8. Polar spongioblastoma, glioblastoma multiforme, oligodendroglioma.

These observations would indicate that although a biopsy of a small amount of tissue may show a relatively benign type of glioma, a more malignant type may be present in other areas.

Discussion

(Dr. William Carpenter MacCarty, Sr., Rochester, Minn.) May I say that this is a very timely and important paper. I do not know the essayists, so I am not throwing bouquets at them, but I see from 1 to 4 gliomas in biopsied specimens every day of the week, and ever since Bailey wrote his textbook on the subject the surgeon has learned it by heart. I go in to report to the neurosurgeon and tell him as little as possible, in so far as names are concerned. I merely tell him it is malignant, or benign, or I do not know. Many of the glorious names have little etymological or practical meaning. I am aware of the fact that what has been said this morning about the practical inadequacy of terminology is absolutely true, so I think it is a timely paper. It may take a long while to educate the surgeon to the point that things are not as simple as they are described in the textbooks.

I also want to congratulate Dr. Edwards on the very beautiful photographs.

ABNORMAL CELL DIVISION AND TISSUE MUTATION (STUDY OF THE DYNAMICS OF MALIGNANT GROWTH). Fritz Levy, Elkins, West Va.

Abstract. Disturbance of cell division, particularly the deficiency in synchronization of the numerous procedures which are correlated, causes characteristic aberrant developments. These are found rarely in "normal" tissue as failure of a single cell. The frequency of such failure increases remarkably in rapidly growing tissue and does so enormously when the rapid growth goes on under very abnormal conditions. In microphotographs of squamous cell carcinoma, adenocarcinoma, osteogenic sarcoma, xanthosarcoma, multiple myeloma, and so on, the same characteristic aberrant development is shown which I described and discussed in previous papers on heteromorphic cells in spermatogenesis as well as in the development of the megakaryocytes of the bone marrow and the fetal liver. I have reported these observations on malignant tumors in various papers since 1920, although briefly. The major portion of this paper, however, is derived from recent American material.

The main difference is that in malignant tumors, observed more often in plants and used in agriculture, areas of cells with changed chromosome content arise, instead of single cells; therefore I speak of "tissue mutations." This term is obviously narrower than the term "somatic mutation," meaning changes in the hereditary mass outside the germ cells. "Tissue mutation" means a change in the hereditary mass of cells with small "prospective potency" or fargone differentiation toward a specially functioning tissue.

A tumor cell is assumed to be a mutation in somatic tissue; that is, an epithelial, or connective tissue, or muscle cell, and so on, which is more or less definitely differentiated as tissue cell, but changed in its hereditary mass, and which propagates, destroying the tissue in which it originates and the surrounding structures, *e.g.* the cell of a primary liver carcinoma produces bile, *i.e.* working as a liver cell, in spite of its otherwise abnormal behavior; just as an albino crow has all the characteristics of a crow except the black

color. For obvious reasons in this analysis only chromosomal mutations are discussed; these are sequels of: (1) loss of one or more chromosomes or parts of them; (2) the contrary, *i.e.* increase in chromosomes, or part of them; (3) polyploidy, including duplication, triplication, and so on, of the normal chromosome set; and (4) poikiloploidy, *i.e.* establishment of a new chromosome set after pluripolar mitosis.

The mutated cells are "asocial" and most of them perish because of incompatibility with the normal tissue. The surviving mutated cells possess sufficient compatibility for life in the individual. "Lethal factors" or "lethal combinations" will prohibit this compatibility or viability. Some "mutations" or tumor forms are found more often than others. It is not very easy to determine from a given tumor when the fatal mitosis occurred, or how far differentiated was the cell concerned. In all probability this cell loses or acquires some qualities, but that does not mean a dedifferentiation or development backward, as the term "anaplasia" is often used (contrary to the definition of its author, Hansemann).

Polyploid or plurivalent giant cells result from atypical mitoses, such as monaster (unicentric mitosis), or nuclear fusion after normal bipolar mitosis without division of the cytoplasm. Subsequent bipolar mitoses in polyploid cells give rise to a "Gigas" tissue, as observed in several tumors; these are secondary mutations in a mutated tissue.

Abnormalities of mitosis are not specific for malignant tumors. Since they are found more often in rapidly growing tissue, they may be used as an indicator for the rate of growth or cell division in the given tissue. Therefore, they are a strong support for the diagnosis of malignancy, when they are found in a heteromorphic tissue, which invades and destroys the normal tissue of the body. The efficiency of the invader depends not only on its own strength, but nearly as much on the relative resistance it meets. Malignancy is a term for an existing abnormal, dangerous correlation.

Discussion

(Dr. Walter M. Simpson, Dayton, Ohio.) From time to time pathologists or surgeons bring to me tissue preparations on which a diagnosis of cancer has been made because a few mitoses were present. One of the things I learned during my fruitful experience with Dr. Warthin and Dr. Weller was that normally proliferating cells often exhibit mitotic figures. The mere presence of mitoses does not mean malignant disease; therefore, I think one sentence by Dr. Levy might well be reemphasized. I believe he said, "Mitoses do not mean malignancy unless they are found in heteromorphic invasive cells."

The Development of Autonomy in Spontaneous Tumors as Indicated by Transplantation Experiments Undertaken at Different Morphological Stages. Harry S. N. Greene, Princeton, N. J.

Abstract. Cancers of the breast and uterus in the rabbit do not arise from sudden transition of normal cells, but, on the other hand, represent the final step in a long developmental process during the course of which the primary neoplastic focus passes through successive stages of anaplastic cellular change, invasion limited to the tissue of origin and eventually of foreign tissue invasion and metastasis. Transplantation experiments undertaken during these various stages showed that the tumors could not be transferred to normal animals previous to the occurrence of foreign tissue invasion, but

could be successfully transplanted elsewhere in the spontaneous host or to animals bearing similar tumors at any stage of their developmental course. A study of the endocrine organs of animals bearing spontaneous tumors indicated an abnormal secretion of estrogenic substances and suggested that this might be a factor essential to the continued growth and development of the tumors. Accordingly, a series of experiments was performed in which dependent tumors were transferred to estrinized animals. In contrast to an early death in normal animals, the tumors grew and underwent progressive development in estrinized animals. It was concluded that one of the factors essential for the continued growth and development of the tumors was an abnormal constitutional status which could be evoked experimentally by the administration of estrone.

An investigation of the factors determining the transplantability of human tumors indicates the existence of a similar developmental course, and further studies are in progress.

Discussion

(Dr. C. V. Weller, Ann Arbor, Mich.) I think this is especially notable in that it gives technics for determining the exact level at which autonomy occurs. It confirms many clinical impressions from the experimental side. The Chair has to restrain himself not to comment in greater detail on this valuable paper.

(Dr. Jacob Furth, New York City.) Have you tried to transplant tumors other than those of the breast? The relation of the breast to estrin is well known. Do estrogens stimulate other tumors? Do other hormones influence the growth of breast tumors in the eye?

(Dr. Greene.) The only spontaneous tumors we have available in any quantity are those of the uterus and the breast, and the only ones worked with in this manner are these. Other hormones have not been tried.

(Dr. Walter M. Simpson, Dayton, Ohio.) This paper has important clinical implications. Geschickter published a paper about 3 years ago (*J.A.M.A.*, 1937, **109**, 1893) in regard to estrogen therapy in so-called "chronic cystic mastitis." I read the paper with some alarm, because I had already observed the most bizarre changes in the breast tissue of women who had been subjected to prolonged and often indiscriminate estrogen therapy. I was pleased to see in the *Lancet* (London, Feb. 5, 1938) a few months later an editorial directing attention to the hazards inherent in Geschickter's recommendation. During the past few months I have examined two women who had received large and repeated doses of estrogen because of the presence of a nodule in the breast thought to have been produced by "chronic cystic mastitis." In both women the most explosive type of acute carcinomatosis occurred with multiple hemorrhagic lesions throughout the breast and axillary tissues. Subsequent radical mammectomy in both cases revealed highly anaplastic, widely infiltrating adenocarcinoma, replacing the regional lymph nodes and filling many of the lymphatic and blood channels with cords of carcinoma cells. There is little doubt that both of these women had small beginning carcinomas of the breast, rather than "chronic cystic mastitis"; neither had been subjected to biopsy. The American Medical Association was recently the defendant in a suit brought by a manufacturer of cosmetics who questioned the right of the Council on Pharmacy and Chemistry to issue a warning to the public against the use of a face cream containing a considerable amount of an estrogen (estradiol). I merely wish to point out that in the present state of

our knowledge the indiscriminate use of estrogen therapy in women with uninvestigated nodules of the breast is a hazardous procedure.

(Dr. Emmerich von Haam, Columbus, Ohio.) I should like to know what dosage of estrin was given.

(Dr. Greene.) I gave the animals two injections a week of 3000 units each, and repeated it for a month, giving a total of approximately 25,000 units.

(Dr. James Ewing, New York City.) I, like our Chairman, am impressed with this remarkable series of experiments. I should like to ask Dr. Greene if he is going to insist on survival in the eye of the animal before admitting autonomy in the tumor growth. That is a pretty severe test. What would have happened if you had transplanted some of those earlier stages of these lesions in a more favorable soil? Would you perhaps not have gotten a greater number of takes and showed a certain amount of autonomy, because in my conception of tumor growth there are all degrees of autonomy. The report on the transplantability of melanoma recalls an ancient observation that melanoma had been transplanted from human tissues into the tissues of lower animals and survived and grown. No one paid any attention to that report, any more than they paid to the similar observation by Moreau in 1894 that he had transplanted tumors successfully from one animal to another.

(Dr. Greene, closing.) In reply to Dr. Ewing's question, it is admitted that the survival and growth of transplanted tissues in the anterior chamber of normal rabbits' eyes is a pretty severe test of autonomy, but as far as I am aware, it is the only practical means of determining the attainment of this state. The anterior chamber of the eye forms one of the most favorable soils for the growth of transplanted tissues and, as a matter of fact, it is the only site in the body that has afforded growth in the case of several of the tumors under study. It was apparent early in this work that different degrees of autonomy characterized different rabbit tumors. In one instance, a Wilms tumor of the kidney, it was found that a period of 6 months was required before the presence or absence of growth in the anterior chamber could be determined, while with other tumors growth is often apparent within 2 weeks. Because of experience with the Wilms tumor, which obviously possesses a very low degree of autonomy, the majority of animals in the present experiment were held under observation for 6 months or more.

SUNLIGHT, SKIN CANCER AND CANCER IMMUNITY. Frank L. Apperly, Richmond, Va.

Abstract. A number of graphs are shown indicating the relationship of total cancer and skin cancer mortalities in the United States and Canada to the solar radiation index and to the number of people exposed to sunlight (percentage of farmers) in each state. In general it is shown that:

1. The apparent discrepancy between those who claim that skin cancer and the general cancer rates vary inversely when different localities are compared, and those who claim a direct relationship between these two forms of cancer is shown to be a matter of climate. In hot climates the relation is an inverse one, in cold climates a direct one.

2. The total cancer mortalities of the various American states and Canadian provinces are shown to fall with increasing solar radiation and with the number of people exposed thereto, and are independent of the production of skin cancer. The fall of skin cancer with increased exposure in cool climates is

merely one example of this general rule. In warmer climates, however, skin cancer may indeed rise in spite of the relatively increased general immunity, *i.e.* the production of skin cancer is not necessary for the appearance of general cancer immunity, as claimed by some observers, but is merely an occasional accompaniment.

Discussion

(Dr. C. V. Weller, Ann Arbor, Mich.) I think this is a very interesting communication. Dr. Apperly has shown the relationship between the total amount of solar irradiation and of skin cancer, and has pointed out certain exceptions to the general positive correlation. I hope as he goes still further with his work he will couple his observations with analyses of the age distribution of the populations concerned, likewise of the racial extractions, because it has been my experience that individuals who are particularly susceptible to cutaneous cancer from solar irradiation fall into a distinct constitutional type. The males particularly are blue-eyed and fair-skinned, and they may be thought of as exhibiting, to a mild degree, the condition of xeroderma pigmentosum. It might, therefore, be a good plan for Dr. Apperly to look into the question whether there is any preponderance of racial types having those characteristics in those northern regions which appear to be exceptions to the general rule which he has established.

(Dr. William Carpenter MacCarty, Sr., Rochester, Minn.) May I ask if Dr. Apperly has taken sun and wind into consideration, because for the last 34 years I have lived in a windy country, and in the winter I get just as much burn as in the summer; wind has something to do with it.

(Dr. Apperly, closing.) That I propose to do, but I have not done it so far.

VISCERAL CHANGES IN THE COURSE OF SINGLE SUBCUTANEOUS INJECTIONS OF BENZPYRENE IN MICE. Rudolf Leuchtenberger (by invitation), New York City.

Abstract. Of a total of 869 white female Rockland mice receiving one injection of 1:2 benzpyrene subcutaneously, 300 were studied histologically. Clinically the mice showed cachexia, edema and paralysis. On histological examination, in addition to the changes described by other investigators (depletion of lymphatic tissue, leukemoid reactions and hepatic lesions), a number of other visceral lesions were observed, particularly in animals autopsied 4 to 8 months after the benzpyrene injection.

1. Twenty per cent of the mice showed more or less extensive perivascular infiltrations without involvement of the vessel wall; 30 per cent presented vessel changes of a severe character in at least two or more organs. These were in the nature of a necrotizing panarteritis, as seen in periarteritis nodosa.

2. More than one-third of the mice presented a diffuse myocarditis with an inflammatory reaction around or within the walls of the coronary arteries and the great vessels at the base of the heart.

3. Hepatic alterations were seen frequently, especially the presence of a diffuse, inflammatory mesenchymal reaction with proliferation of the reticuloendothelial cells, which in some instances showed a tendency to invade the walls of large vessels, suggesting a transition to neoplasia.

One hundred normal control mice showed only slight changes or no changes at all.

Discussion

(Dr. Harold L. Stewart, Bethesda, Md.) I can confirm many of the observations that have been reported in mice treated with carcinogenic hydrocarbons in the work we have done at the National Cancer Institute. However, in our experience we have seen a higher incidence of similar lesions in our control mice than has been reported in this series of experiments.

A Study of the Black Pigment Found in the Human Lung. C. E. Woodruff and (by invitation) Georgine A. Moerke, Northville, Mich.

Abstract. During the nineteenth century the nature of the black pigment found in the human lung was the subject of considerable discussion. However, at the present time it is generally accepted that the black material found in the lungs and bronchial lymph nodes of most adults is the residue of inspired smoke, soot or coal dust.

In a series of 20 cases black pigment was isolated from human lungs by digesting the tissue in concentrated hydrochloric acid for a period of 4 weeks. At the end of that time the insoluble residue was washed repeatedly in normal hydrochloric acid. In a given case the yield of pigment was what one might expect from the gross appearance of the organ: a lung that was black throughout might give as much as 10 gm. of soft, slimy black residue, while a light gray lung would yield only minute amounts of pigment. The pigment samples have been subjected to the following tests:

1. When placed in 1 per cent sodium carbonate a portion of the pigment goes into solution; in 10 per cent sodium carbonate the pigment swells and forms a fluffy stringy mass which will not sediment on standing.

2. When subjected to an organic solvent such as methyl alcohol a major portion of the pigment goes into solution leaving, as a final residue, a gray to black material. Under the microscope this material shows doubly refractive particles, presumably silica, and a few, relatively large, angular black particles which are probably bits of charcoal or coal. These latter form an insignificant part of the original pigment mass.

3. When placed in an alkaline chlorine solution such as Dakin's, the pigment slowly bleaches — in some cases to a snowy white, in others to a light gray.

4. Other tests indicate the presence of a phenolic group in the pigment molecule.

5. Analysis reveals a nitrogen content of 4 to 5 per cent on an ash-free basis.

The carbons which we have used as control substances — lamp black and hard and soft coal dust — have proved inert when subjected to these various tests.

Anatomically the lung pigment is commonly found in association with chronic tuberculous processes and with silicosis. Our blackest lung came from a patient with silicosis who worked in the iron and copper mines of upper Michigan. Our various tests indicate that the major portion of lung pigment is not ordinary carbon but instead a phenolic, nitrogen-containing compound, endogenous rather than exogenous in origin.

Discussion

(Dr. Ralph D. Lillie, Washington, D. C.) I have been disturbed recently by a tendency in some cases, particularly in animals, to stain with basic dyes in

these dark colored pigments in many lungs, and this might afford some partial explanation. I have thought, as the rest of us have thought, that we were looking at carbon when we saw specks of black pigment.

(Dr. M. J. Shear, Bethesda, Md.) For a number of years the U. S. Public Health Service has been interested in the organic material contained in dust particles present in the air. We have collected specimens of dust from ordinary street air and upon extraction with organic solvents such as benzene or ether we have obtained black tarry material which does not contain insoluble carbon. That is, if these extracts are filtered through filter paper, leaving behind insoluble carbon and carbon compounds, a solution is obtained which upon removal of the organic solvent gives a black tar.

I should like to ask the preceding speaker whether he has tested in the same way as he has examined the tissue from the lung, not carbon black but the organic compounds which are present in the air. I am wondering whether this needs to be done in order to ascertain whether the black material in the lungs, which is decolorized by his procedure, is of exogenous or endogenous origin.

(Dr. Arthur J. Vorwald, Saranac Lake, N. Y.) We have been concerned with this problem in our study of pneumoconiosis and silicosis, but we have been unable to find a method which effectively differentiates between an endogenous and an exogenous pigment. This has been of particular concern to us in studying the iron pigments in men who have worked in iron mines where we are called upon to differentiate between hemosiderin from the breakdown of red blood corpuscles and the inspired oxides of iron which those individuals might have inhaled. We have not as yet developed a method by which we can differentiate between an endogenous and an exogenous pigment, whether it be in the form of carbon or of the oxides of iron.

(Dr. Woodruff, closing.) The controls which we have had available have been lamp black and various coal dusts from soft and hard coal. It is possible that there are tarry materials present in the air of cities which might be a factor in explaining the pigmentation of the lungs.

With regard to iron in the pigment, our tests for iron pigment have been negative.

CHEMICAL ISOLATION AND FRACTIONATION OF AMYLOID. George M. Hass, New York City.

Abstract. Amyloid-bearing tissues obtained postmortem from patients with chronic pulmonary tuberculosis were cut into thin sections with a freezing microtome. Three types of amyloid were observed. All were insoluble in buffer solutions ranging from pH 1 to pH 10. The three varieties, whether present in the liver, spleen or kidney, were soluble at 5 to 10 C. in phosphate buffer solution, pH 11. Almost all normal liver protein soluble in the range pH 7 to pH 11 was soluble in the range pH 7 to pH 10. By successive extractions of thin sections of fresh amyloid-bearing liver at pH 7, pH 10, and finally at pH 11, it was possible to obtain hepatic amyloid protein in a reasonably pure form. Two fractions of protein were obtained. The principal fraction amounted to 85 to 90 per cent of the total protein extracted at pH 11. It remained in solution upon neutralization of the solvent. It began to precipitate at 15 to 17 per cent saturation with ammonium sulfate, and was completely precipitated at half saturation with ammonium sulfate, by addition of acetic acid, or by dialysis against distilled water. The second protein

fraction remained in solution upon neutralization of the alkaline buffer solvent. It was precipitated along with the principal fraction by half saturation of the solvent with ammonium sulfate, but was soluble in dilute acetic acid and distilled water.

Elementary analysis of the principal protein fraction is as follows:

C = 51.48
H = 6.84
N = 16.41
P = 0.01
S = 0.72
SO_4 = 0.08

Discussion

(Dr. C. V. Weller, Ann Arbor, Mich.) Of very great clinical-pathological importance is the question whether amyloid is reversible or not. I should like to know whether Dr. Hass' investigations of the chemistry of amyloid throw any light on that question.

(Dr. Hass, closing.) I do not believe I can answer that question.

ANISOTROPIC CRYSTALLOIDS IN THE HUMAN THYROID GLAND. Maurice N. Richter, New York City.

Abstract. Examination of microscopic sections of human thyroid glands in polarized light with crossed polarizer and analyzer shows, under certain conditions, an anisotropic crystalline-like material in the colloid. Only occasional reports of such material are found in the literature, and no previous observations have indicated a correlation with clinical or histological features. The material has not been found in the thyroid glands of lower animals.

The anisotropic material is in the form of minute colorless particles of different sizes and various forms which, with crossed prisms, become alternately light and dark as the object stage is rotated through 90 degrees. Although visible in fresh teased specimens, the field is usually obscured by the presence of other anisotropic substances.

The particles are soluble in dilute acids but not in organic solvents, and therefore remain after the usual embedding methods if acids are avoided in fixation. They are unstained by the ordinary histological dyes, but are blackened by silver nitrate (the Kóssa reaction) and by Millon's reagent. Microincineration also causes the material to become black but not to disappear. These reactions and solubilities suggest that the material is a calcium compound.

The incidence of the material was studied in a series of 443 thyroid glands examined at autopsy and 104 glands removed surgically. Because of unequal distribution of the material, the glands were arbitrarily classed in only two categories: those with little or no material, and those with a moderate or large amount. In the glands removed at autopsy there is a tendency for the proportion in the latter group to increase with age. This tendency is greater among normal than among abnormal (hyperplastic, involuted or nodular) glands. The proportion of normal glands having moderate or large amounts of anisotropic material is lower in the fifth decade than in the fourth or sixth. This drop of incidence is observed among normal glands of both males and females, but not among glands with hyperplastic or nodular areas.

In general, the proportion containing moderate or large amounts of anisotropic material is greater among normal than among abnormal glands of the same age groups. In diffuse or local hyperplasias the material is usually absent, and in nodular glands it is unequally distributed among the nodules. Occasionally no anisotropic material is seen in the nodules, although moderate amounts may be found in the surrounding areas.

There is thus a correlation between the presence of the anisotropic material and the age of the individual, as well as the functional state of the gland.

THE CYTOLOGICAL RESPONSE IN THE RAT TO STREPTOCOCCUS VIRIDANS. Paul Gross, Frank B. Cooper and (by invitation) Jane D. Phillips, Pittsburgh, Pa.

Abstract. Single or repeated intracardiac injections of living *Streptococcus viridans* cultures in rats resulted in valvulitis of the atrioventricular as well as of the aortic valves. The valvulitis which followed multiple infections was at times characterized by extensive and bulky vegetations. Focal collections of cells resembling those in Aschoff bodies were found in interstitial and occasionally in perivascular positions in the ventricular myocardium of rats given four or more infections over periods of 1 week or more. These cellular foci did not contain the collagenous fragments or the reticulum considered characteristic of the Aschoff body. Rats given only a single infection did not exhibit such cellular foci.

Nearly all animals, whether subjected to single or multiple infections, showed focal embolic glomerulonephritis. Rats which were infected four or more times showed foci of large mononucleated phagocytes in the brain and the lungs. In the latter situations the foci were sharply circumscribed in the form of miliary nodules and the cells were frequently fused as the Langhans type of giant cells. Animals that had received only a single infection did not exhibit such foci.

Discussion

(Dr. Ward J. MacNeal, New York City.) I wish to congratulate the authors on their successful production of cardiac vegetations in rats. However, one has to recognize that the injury to the heart by the introduction of the needle into that organ complicates the problem of utilizing such material for further experimental purposes. We are not quite sure how much trauma has been produced by the needle. The appearance of the pericardial lesion is probably due to the local trauma to a large extent, because we have not observed this when we produced endocarditis by injection into the ear vein of the rabbit. There is a distinct advantage, of course, in being able to use smaller animals, from the economic point of view. If one can utilize rats and mice instead of rabbits that is quite a saving, but their hearts are smaller, which is somewhat of a handicap. I believe that in the future we shall be able to produce *viridans* lesions in a great variety of animals. We have been able, with Dr. Ferry, to produce such lesions in the endocardium of a horse, so that it is quite possible that *viridans* endocarditis may be produced in many different animals.

(Dr. Gross, closing.) In answer to Dr. MacNeal's comment, I think his statements regarding the rôle of trauma are in order. However, we made no claim as to the endocarditis. We merely wish to call attention to the type of

lesion produced in the myocardium, which has been described by a number of workers in other animals. Some have claimed these lesions to be Aschoff bodies, while other workers have insisted that these lesions, produced mostly in rabbits, were not Aschoff bodies.

ANATOMICAL CHANGES PRODUCED BY SHORT PERIODS OF ANOXIA (ANOXIC SHOCK). THE EFFECT OF FREQUENT REPETITIONS AND COMBINATION WITH INSULIN. Joseph Tannenberg, Albany, N. Y., and New York City.

Abstract. The term anoxic shock was employed for the sake of brevity to denote a short period of increasing anoxia which reached its peak in a state of gasping respiration or with arrest of respiration within a period of from 15 to 30 minutes. It was produced by flowing mixtures of oxygen and nitrogen. Anesthetics were intentionally not employed, and carbon dioxide accumulation was prevented. A total of more than 350 shocks was administered to 38 rabbits, 15 of which were sacrificed within the first 3 days, 7 between the 4th and 14th day, 9 between the 16th and 25th day, and 7 between the 46th and 61st day, after having been subjected to from 1 to 4, 4 to 19, 4 to 51, and 8 to 18 anoxic shocks respectively. A few additional rabbits were continuously subjected to severe anoxia for 2, 3, 7 and 8 hours on one single day.

Previously healthy rabbits withstood a single anoxic shock well, even if it were extended to arrest of respiration. Short artificial respiration was sufficient to revive such rabbits. No morphological changes were demonstrable in rabbits sacrificed at the end of the first shock, or when severe anoxia had been maintained for several hours continuously. If such rabbits were sacrificed 24 hours later, slight edematous focal changes were found in the cardiac musculature, especially within the posterior wall of the left ventricle. They were reversible within a few days, and did not produce cellular reaction or scarring. When anoxic shock, however, was extended to the peak and repeated several times on the same day with intervals of about one-half hour in between, or in a series of more than 6 or 7 successive days, real focal necroses were produced in the cardiac musculature, the skeletal musculature and the liver, aside from severe reversible and irreversible degenerative changes that involved single or small groups of ganglion cells in many parts of the brain, especially in the area beneath the floor of the fourth ventricle, among the Purkinje cells in the cerebellum, in the basal ganglia and Ammon's horns.

When anoxic shock was administered daily for about 1 week the same rabbits that had previously withstood oxygen levels of 6 or 7 per cent became exhausted at a level of 10 or 12 per cent. This was an unmistakable sign that 1 day's rest is too short a period to restore to normal even the slight changes produced by a single anoxic shock. The cumulative effect on the heart of anoxic shock frequently repeated with too short intervals in between was impressively demonstrated by the sudden development of fatal pulmonary edema in the gasping state of respiration. This occurred unless the shock was immediately interrupted at the onset of this state, the approach of which was indicated by a definite reduction of the respiratory rate. Normal rabbits sacrificed by suffocation in a nitrogen atmosphere for control exhibited well collapsed lungs in contrast to the overextended edematous lungs of these rabbits.

The combination of insulin and anoxic shock was studied in 14 additional

rabbits. Insulin was injected subcutaneously 3 to 5 hours prior to anoxic shock. With such timing insulin definitely enhanced the effect of anoxic shock. Insulin doses which lowered the blood sugar level not more than to 50 to 60 mg. per cent merely enhanced the sensitivity of the rabbits to anoxic shock without increasing its danger. Large doses of insulin, 2 units or more per kilo body weight, however, definitely increased the danger of anoxic shock. The respective rabbits reacted similar to those that had been exposed previously to many anoxic shocks, and they died of pulmonary edema unless anoxia was terminated immediately at the first gasping inhalation.

PATHOGENESIS OF IRRADIATION SICKNESS: A NEW METHOD FOR INDUCING SHOCK. Virgil H. Moon, Karl Kornblum (by invitation), and D. R. Morgan, Philadelphia, Pa.

Abstract. Exposure of the abdomen to deep roentgen irradiation is followed by physiological disturbances similar to those of shock resulting from other causes. Such irradiation damages the intestinal mucosa, resulting in delayed necrosis. The resulting signs of illness are likewise delayed in onset. This is accompanied by progressive hemoconcentration. The visceral findings post-mortem are those characteristic of shock regardless of its origin.

Abdominal irradiation provides a new method for inducing shock by damage to tissues. This method eliminates pain, emotional reactions, sympatho-adrenal hyperactivity, hemorrhage and anesthesia, all of which have been assigned as causes for shock by some writers.

Discussion

(Dr. Harold W. Jacox, Pittsburgh, Pa.) May I point out that the amount of irradiation which was used in these dogs must have been enormous, because we have never seen any such shock produced by human therapeutic exposures. I would like to have Dr. Moon tell us what amount of irradiation was given. I have no doubt that shock can be produced in animals by irradiation, but I think the amount given must have been massive.

(Dr. Moon, closing.) The presentation was introduced by the statement that massive irradiation applied to the abdomen produces these effects. Such effects in human beings have been reported many times when massive irradiation was given for therapeutic purposes to induce regression of neoplastic growths involving the abdominal region. The dosage used in these animals was 130 kv., 5 milliamperes, 12 inch target distance, with a 2 mm. aluminum filter over the abdomen to prevent superficial burning. The roentgen units employed ranged between 1400 and 2800. It was found that about 1800 r units under the conditions mentioned would produce the effects described, and this was regarded as the approximate minimal lethal dose in such experiments.

LATE PATHOLOGICAL EFFECT OF HIGH VOLTAGE X-RAYS UPON THE HUMAN LUNG. John T. Bauer and (by invitation) Paul H. Schraer, Philadelphia, Pa.

Abstract. Two cases are presented to show the late effect of X-ray irradiation upon the lung.

Case 1. An Italian male, 53 years of age, with a clinical diagnosis of carcinoma of the lung, received 8800 r units of high voltage X-rays over the chest. Two months after the last treatment irradiation pleuropneumonitis

was noted. Progressive massive fibrosis led to his death 2 years later after a severe attack of dyspnea. At autopsy pleural thickening and a sharply defined pulmonary fibrosis of the upper lobes resulted in a distortion of the trachea and a marked contraction of the lung. Death was due to pulmonary thrombosis.

Case 2. A Greek male, 54 years of age, with a carcinoma of the bronchus and bronchiectasis, received 9000 r units of high voltage X-rays over the chest. Subsequent roentgen examinations showed a progressive irradiation fibrosis. Death occurred after a severe attack of dyspnea 3½ years later. At autopsy, in addition to changes similar to those in Case 1, the entire right lower lobe of the lung consisted of loculated and bronchiectatic cavities. There was no gross evidence of tumor.

Microscopically the features were: (*a*) a disappearance of alveoli, except for residual alveolar and bronchial spaces lined by altered bronchial epithelium; (*b*) squamous metaplasia of the bronchial epithelium; (*c*) transformation of the lining cells of the residual alveolar spaces to a low columnar or a cuboidal type, frequently with a many layered proliferation of epithelial cells; (*d*) proliferation of the intima of small arteries; (*e*) hemorrhages in and about the walls of blood vessels; (*f*) progressive interstitial fibrosis with obliteration of alveoli; and (*g*) condensation or proliferation of elastic fibers within the parenchyma into small balls or masses, and fraying of elastic fibers within the vessels. A low grade chronic inflammation consisting chiefly of lymphocytes and macrophages extended throughout. Nothing attributable to irradiation was observed in the other organs.

Both patients showed a progressive pulmonary fibrosis. Both had antecedent disease of the lung. While the changes could be the result of the combination of tumor, infection and irradiation, since similar changes have been produced in animals by X-rays alone, it is possible that the changes we have described in man may be due entirely to irradiation.

Discussion

(Dr. Shields Warren, Boston, Mass.) I am inclined to agree with Dr. Bauer, on the basis of a series of several hundred cases of irradiated chests which we have had the opportunity of examining at autopsy, that these changes are due to irradiation. I think a very important factor, however, is the presence of atelectasis and infection in these cases. One of the early effects of radiation is, as Dr. Bauer has pointed out, the loss of the normal ciliated bronchial epithelium, which makes for a damming up of secretion, and the establishment of infection throughout the lung. This type of change leads to the fibrosis which he has shown. I feel that this can be better substantiated by some of our cases than by his, since some of ours were irradiated for a carcinoma of the breast overlying the thorax, when there was no pathological process presumably present in the lungs, although metastases invisible to X-ray might have been present in such cases. The dosage is practically always heavy in these cases. I wish that Dr. Bauer had shown a higher power microphotograph than he did, because many of the alveolar cells as a result of irradiation show a rather characteristic increase in size and irregularity in nuclear appearance.

(Dr. W. C. Hueper, Scarsdale, N. Y.) The findings which Dr. Bauer reported are substantiated by the occurrence of pulmonary fibrosis in workers in radium laboratories in Germany. There are several cases of that type on

record which are identical in histological appearance with the changes Dr. Bauer has described.

In regard to the atypical appearance of the epithelial cells, in these same laboratories there have also occurred several cases of pulmonary cancer suspected to be of occupational origin.

(Dr. James Ewing, New York City.) Dr. Bauer is dealing with a very acute problem in radiation therapy. I think we would know more about what happens in these lungs if we had more data on the dosage. He stated that 4000 r was given in 1 case, and 9000 in another. Those figures do not mean anything unless one specifies the factors. The severe effects depend on the portal as much as on the r dosage, and they can generally be avoided in the treatment of lung tumors by reducing the portal and by limiting the X-ray to the tumor areas. Also, in the treatment of mammary cancer by limiting the portal and by tangential radiation one can eliminate most of these evidences of damage to the lung. However, our experience has suggested that in all probability the lungs that develop postradiation changes were not normal lungs originally, because identical doses can be entirely harmless to one person and cause a severe reaction in another. I am inclined to think there is some underlying condition of the lung which renders it more susceptible in one individual than in another.

I should like to report on the further ill effects of very severe radiation of the lung in a patient with carcinoma who survived — let me say survived rather than was cured — for a period of 11 years following radiation. The patient died from complete collapse and atrophy of the affected lung, closing of the pulmonary artery on the affected side, and an enormous dilatation of the right auricle, which almost reached about 10 cm. in diameter, and death resulted from the circulatory disturbance. I wonder what will happen to some individuals with total pneumonectomy if they survive 11 years — if there will be a readjustment of the circulation to make good the loss of one pulmonary artery.

(Dr. Bauer.) I do not believe I can answer the question by Dr. Warren as to the cytological changes in the alveolar epithelium; I did not make careful observations on it but I certainly will, now that my attention has been called to it.

Dr. Ewing is correct when he says it is important to know the dosage and all the factors regarding the radiation which the patient received. I did not wish to go into those details because of lack of time, but I will be glad to mention them now. The first patient received 4400 r units over a period of 3 to 4 weeks, through each of 2 portals, 1 anterior and 1 posterior. The factors were 200 kv., 50 cm. target distance, portals 15 by 20 cm., filtration 1 mm. aluminum and 0.5 mm. copper. In the other case I am not sure as to the size of the portals; I think each was 20 cm. in diameter, again anterior and posterior, with a total of 9000 r, 4500 r anterior and 4500 r posterior, all the other factors being the same as mentioned above.

(Dr. Ewing.) Those are very large portals, are they not?

(Dr. Bauer.) Yes, but as the lesion was considered to be quite large, a large dose over a wide area was desired.

(Dr. Ewing.) Have you any evidence bearing on the previous integrity or abnormal condition of the lungs that react in this manner?

(Dr. Bauer, closing.) I have no immediate evidence, except for my reading of the literature. There are a number of authors who suggest that there

is an unusual susceptibility to irradiation because of infection or tumor. Engelstad's work in Europe was very interesting to me because he could produce similar changes in the lungs of rabbits without any infection or evidence of tumor by irradiation alone.

HISTOLOGICAL VISUALIZATION OF VITAMIN A IN HUMAN ORGANS UNDER NORMAL AND PATHOLOGICAL CONDITIONS. Hans Popper (by invitation), Chicago, Ill.

Abstract. Vitamin A is dissolved in the body lipoids in various concentrations. Its presence causes a striking greenish fluorescence of these lipoids which fades during observation under the fluorescence microscope because of irradiation with ultraviolet light (Querner). This characteristic fluorescence permits the microscopic and photographic visualization of vitamin A in tissues. As evidence for the specificity of the fluorescence for vitamin A the following facts are offered: (1) the presence of the same fluorescence in vitamin A concentrates; (2) the identical distribution in the body of the fluorescence and vitamin A as determined chemically; (3) similar histochemical reactions of vitamin A concentrates and fluorescing inclusions; and (4) experiments on vitamin A deficient rats with repletion.

In the human liver the fluorescence is found in Kupffer cells, in fat droplets, in small acetone-soluble droplets not giving ordinary fat reactions, in lipofuscin and diffusely dispersed in the cytoplasm. There is great variety in amount and distribution. In adults there is no striking reduction except in damage to the liver; in infants only small amounts are visible. In damage of slight degree to the liver the liver cells are free and the Kupffer cells rich in the fluorescence. In more severe damage the Kupffer cells are free also, as in a Zahn infarct, or in decompensated cirrhosis or hepatitis. In the latter not only the liver but also other organs are poor in vitamin A, indicating an increased use of the vitamin under these conditions, in addition to the inability of the liver cells to retain it.

In the adrenal of the adult it is found in the fascicular and glomerular layer. It is reduced in infectious and exhausting diseases, or in damage to the liver. The fluorescence is absent in infants up to the second month.

In the testicle vitamin A is found in the tubular and Leydig cells, superimposed in the latter over a brown fluorescing substance visible after fading of the vitamin fluorescence. Both appear only after puberty and disappear at higher ages and in exhausting diseases. In the corpus luteum of the ovary it is found in small droplets in the granulosa cells and in larger amounts in the theca cells; in the latter it is superimposed over a brownish substance. After dissolution of the corpus luteum the vitamin-carrying theca cells remain scattered over the stroma, but disappear during the climacterium.

The normal kidney does not show vitamin A; in nephritis it occurs in the tubules and the interstitium. Non-lactating breast tissue fails to show it; the lactating glands are extremely rich in it. The fat tissue shows only small amounts.

Histological demonstration may throw some light on the rôle of vitamin A at the present time. It need not be present in the epithelium. It is absent in the epithelium of the cornea, skin, teeth or gastro-intestinal tract, except during resorption, and positive chemical tests may be due to vitamin in fat cells in the submucosa. Since the first signs of vitamin deficiency appear in

the epithelium of these organs, a distant effect is to be assumed. Vitamin A has some relation to fat metabolism. Its presence may differentiate a normal fat storage, as infiltration, from a pathological degeneration. A relation to the sexual functions is indicated by the fact that it is visible in the cells which are considered to be the site of the production of the sex hormones.

Discussion

(Dr. Alex B. Ragins, Chicago, Ill.) On the basis of the experimental work of Dr. Popper and the demonstration of vitamin A under the fluorescent microscope, Dr. Popper and I have studied a number of benign and malignant tumors of various organs of the body. We have found a fairly good parallelism existing between tumors arising from vitamin A-containing tissues and those arising from non-vitamin A-containing tissues. In the former the tumors frequently contained vitamin A, and in the latter the greenish fading fluorescence was absent. I should like to mention some of the types of tumor containing vitamin A. Vitamin A was present in considerable amounts in tubular adenomas of the breast and in small quantities in plant cells of anaplastic carcinomas of the breast, whereas it was absent in peri- and intracanalicular fibroadenomas. Vitamin A was demonstrated in the fat-containing carcinomas of the prostate. It was also present in cortical adenomas of the adrenal. In hypernephromas the vitamin A content was considerable. The tumor cells were arranged in cords resembling the fascicular layer of the adrenal cortex. In places the papillary processes of the tumor likewise contained tumor cells rich in vitamin A, and since the papillary processes are neither characteristic of the normal kidney nor of the adrenal gland, the presence of vitamin A supports the supposition that hypernephromas arise from adrenal rests. In 2 cases of papillary adenoma of the renal cortex no vitamin A was found.

An interesting observation was made concerning granulosa cell tumors of the ovary. In this tumor we found two types of cells containing vitamin A; one resembling the luteinized granulosa cell containing no doubly refractile bodies, and the other resembling the luteinized theca cells containing doubly refractile bodies.

Vitamin A was found in small to moderate amounts in dysgerminomas of the ovary and the testicle. In a malignant teratoma of the ovary we found vitamin A in fat cells and in a few, immature, plant-like epithelial cells.

I might add that chemical analysis was made of the more viable parts of some of the tumors, particularly the granulosa cell tumors. Extracts of these tumors gave a positive Carr-Price reaction for vitamin A.

THE PATHOLOGY OF ACUTE AND CHRONIC THIAMIN DEFICIENCY IN THE PIGEON.* Roy L. Swank (by invitation), Boston, Mass.

Abstract. A correlation of the histopathology and clinical behavior of thiamin-deficient pigeons has been undertaken. Opisthotonos in acutely thiamin-deficient pigeons was frequently unattended by degenerating nerve fibers or neurons in either the central or the peripheral nervous system. When the deficiency was complicated by starvation it developed more slowly, opisthotonos appeared later, and many degenerating nerve fibers were usually present. In both instances the opisthotonos disappeared in a very short time after thiamin was injected intramuscularly.

* Presented by Dr. S. B. Wolbach in Dr. Swank's absence.

A more chronic deficiency, characterized by weakness of the legs (opisthotonos being absent) appeared when the ration was partially deficient in thiamin, or occasionally when the caloric intake was grossly inadequate. In birds of this type degenerating nerve fibers were always found in the peripheral nerves. The number of such fibers in the sciatic nerves corresponded closely with the degree of the paralysis, and during repair, which occurred when thiamin (irrespective of other factors) was added to the ration, nerve fibers regenerated (increased in number) at a rate which paralleled the clinical improvement closely. The large and long nerve fibers, many of which could be traced directly into the dorsal ganglia, degenerated first, and if the deficiency were prolonged, smaller nerve fibers became affected as well. In many of these pigeons with marked weakness of the legs cell bodies in the dorsal ganglia exhibited lysis of chromatin and eccentricity of the nuclei. This was observed nowhere else in the nervous system. During repair and until after complete recovery from paralysis, these phenomena (chromatolysis) persisted.

In chronically thiamin-deficient pigeons large and long degenerating nerve fibers were found in two regions of the spinal cord at all levels. One group of these in the ventral funiculus was thought to arise in the reticular region of the medulla oblongata; the other, which was situated in the posterior part of the lateral funiculus, could be followed to the lateral surface of the medulla oblongata and from there by way of the inferior cerebellar peduncles into the medullary portion of the cerebellum. In the central as well as the peripheral nervous system the long and large nerve fibers degenerated first. Medium and small sized fibers were affected later so that the degeneration became quite generalized.

In many of the chronically deficient pigeons with weakness of the legs incidental lesions compatible with cardiac failure were found at autopsy. In the hearts from many of these pigeons microscopic examination revealed many areas of focal necrosis, some of which had become infiltrated with polymorphonuclear leukocytes.

In a peripheral neuron of a thiamin-deficient pigeon the first consistent morphological alteration appeared in the axis cylinder. No doubt a period of functional impairment of the neuron (such as produced opisthotonos) preceded this. The axis cylinder followed by the myelin sheath degenerated at a point most distal to its trophic cell body for a variable distance, depending on the severity and duration of the deficiency. The cell body (in a dorsal ganglion) appeared to shrink first and later exhibited chromatolysis. When thiamin was administered the axis cylinder (and myelin sheath) regenerated, and when this was complete the cell body returned to normal.

It has been concluded that the opisthotonos of thiamin deficiency is a manifestation of decerebration due to a functional impairment of the neurons of the higher cerebral centers. Weakness of the legs, when produced by the same deficiency, is due to degeneration of peripheral nerve fibers within the sciatic nerve. Heart failure may be attended by no visible histological changes, but in many instances necrosis of myocardial fibers occurs.

Discussion

(Dr. Paul Klemperer, New York City.) Can you tell us what the changes in the heart are. Are they identical with those in beriberi?

(Dr. Wolbach, closing.) Yes; they have hydropericardium, and in the extreme cases they have actual necrosis of muscle fibers.

SATURATION TESTS IN THE DETERMINATION OF VITAMIN C IN THE HUMAN. George J. Kastlin and (by invitation) Clara R. Schlesinger, Pittsburgh, Pa.

Abstract. In the study of vitamin C metabolism a desirable method must give information as to the available store of the vitamin in the body.

An intravenous test dose of 500 mg. of cevitamic acid will produce variations of the blood level and an acceleration of urinary excretion during a 4 hour period of observation, from which the degree of saturation of the vitamin C can be determined. This test is adaptable to ambulatory as well as hospital patients. The following test was used: (1) collect the fasting blood sample and specimen of urine to determine the mg. per cent of cevitamic acid of the blood plasma and urine; (2) give 500 mg. of cevitamic acid intravenously; (3) determine the mg. per cent of blood vitamin C at 5 minutes, 1, 2, 3 and 4 hours. Collect total urine at 1, 2, 3 and 4 hours; (4) the curve of mg. per cent of blood cevitamic acid and milligrams excreted in the urine are plotted. In addition the total urinary excretion is computed.

Interpretation of the saturation test is based on: (1) height of fasting blood level; (2) height of 5 minute blood level; (3) rate of fall of blood concentration from 5 minute peak to the 4 hour level; (4) height of 4 hour blood level in relation to the fasting blood level; and (5) the total percentage of test dose excreted in the urine.

The normal saturation curve: (1) the fasting blood level is 0.7 mg. per cent of cevitamic acid or more; (2) the 5 minute peak is usually between 4.5 mg. to 0.6 mg. per cent; (3) the rate or speed of return is gradual to a 4 hour level above the fasting level; and (4) the total urinary excretion is 40 per cent or more of the test dose.

In marked vitamin C deficiency: (1) the fasting level is below 0.4 mg.; (2) the 5 minute peak is slight or variable; (3) there is a rapid fall of blood concentration to a 4 hour blood level at or below the fasting level; and (4) the total urinary excretion is below 20 mg.

Extreme depletion of vitamin C stores of the body may be present with no evidence of clinical scurvy. The fasting blood level alone or the relationship of the 4 hour level to the fasting level is not an adequate guide to saturation. Urinary excretion is related to the height of fasting blood level to a degree in patients with a stabilized vitamin C store. In fluctuating vitamin C saturation the relationship of fasting and 4 hour blood levels to urinary excretion is variable.

During a period of increasing saturation increase in total urinary excretion and elevation of the 4 hour blood level above the fasting level occur before the fasting level is stabilized at a normal range. The reverse is true in the process of desaturation. The hourly collection of urine is of value to determine alterations of kidney clearance of vitamin C.

A better understanding of the nutritional state of the patient is possible by considering all of the factors discussed above.

ASCORBIC ACID (VITAMIN C) AND THE ANAPHYLACTIC REACTION IN GUINEA PIGS. Malcolm H. Soule and (by invitation) Helen Loomis Gehring, Ann Arbor, Mich.

Abstract. A group of 150 male guinea pigs weighing approximately 250 gm. each was treated as follows: 50 received 1 mg. of l-ascorbic acid in 0.1 cc. of PSS subcutaneously at approximately the same time each day for 3 weeks; an equal number received 10 mg. of the acid daily; and the others served as

controls. At the end of this period the two treated groups were divided and representatives of each were sensitized with fresh egg white, horse serum and cow serum by the intraperitoneal injection of 1 cc. of a 1:100 dilution of the antigens. The administration of the ascorbic acid was continued as before for an additional 3 weeks when a shocking dose of 0.5 cc. of a 1:10 dilution of specific antigen was introduced into the leg vein of each animal. Typical anaphylactic reactions were elicited in each instance. The controls were of two types: one group of animals received 0.1 cc. PSS daily for 6 weeks, the other PSS daily for 3 weeks, then sensitizing doses of the three antigens followed by PSS injections for an additional 3 weeks. At the end of the 6 weeks, shocking doses of the specific antigens were injected. The non-sensitized animals gave no reactions; those that were sensitized responded typically to the antigens. On the basis of these findings it has been most difficult to reconcile the diversity of results reported in the literature relative to the influence of ascorbic acid on the anaphylactic reaction in guinea pigs.

Discussion

(Dr. Walter M. Simpson, Dayton, Ohio.) At the Kettering Institute we have been engaged for several years in an investigation of the suppression of the anaphylactic reaction. Our interest was aroused by the fact that we had observed that certain of the allergic phenomena were minimized or suppressed by fever. It is an old and not infrequent clinical observation that many persons with severe allergic phenomena improve or are cured by an illness accompanied by high fever. We were first struck by the fact that patients who were highly sensitized to arsenical drugs could tolerate large amounts of these drugs, such as arsphenamine, if the arsenical compounds were administered intravenously at the height of induced fever. In collaboration with workers at the University of Cincinnati and at the Michigan State Laboratory at Lansing, it has been found that guinea pigs which were sensitized to horse serum and then given the shocking dose of horse serum 1 week later, during an induced fever of 107 F. (41.7 C.), did not develop anaphylactic shock; all of them survived. The sensitized control animals, not subjected to the fever but given an identical shocking dose of horse serum, died promptly in anaphylactic shock.

(Dr. E. R. Long, Philadelphia, Pa.) There is another type of sensitization which is said to be affected by vitamin C more than the anaphylactic type. That is the tuberculin type, in which the capillaries are involved. I wonder if Dr. Soule has had any experience with that type of sensitization.

(Dr. Leo Alexander, Boston, Mass.) I am very much interested to learn that Dr. Soule and Dr. Gehring were able to exclude vitamin C as a factor of influence in the intensity of the anaphylactic reaction. I have never studied this problem scientifically, but from a simple empirical point of view I am under the impression that guinea pigs fed a diet that does not contain fresh vegetables can be sensitized more easily and show a more striking local anaphylactic reaction than those that receive green vegetables as a regular part of their diet. I should like to ask Dr. Soule whether he considers it possible that there may be a factor other than vitamin C in green vegetables which may be responsible for lowering the intensity of anaphylactic reaction. I should like to ask Dr. Soule whether he had a control series of animals fed a diet with and without green vegetables similar to his series of animals with and without vitamin C.

(Dr. Soule, closing.) In answer to Dr. Long's question, the only work I know of along that particular line was reported by Dr. Steinbach before the Third International Congress of Microbiology in New York in September, 1939.

I might say in answer to Dr. Alexander's comments that we attempted to maintain these guinea pigs on an adequate diet, but the injection of vitamin C gave us much better looking animals at the end of the experiments than the controls. I cannot answer your question.

RICKETS: PAPPENHEIMER'S 84 DIET AND ITS EFFECT UPON THE GASTROINTESTINAL MUCOSA OF THE RAT. N. W. Popoff, Rochester, N. Y.

Abstract. On investigating the origin and functional significance of the argentaffin cells of the gastrointestinal tract (*Arch. Path.*, 1939, **27**, 841) the conclusion was reached that argentaffinity represents a specific phase in the cytomorphosis of the mucous and parietal cells, and that this cytomorphosis signifies rejuvenation of functionally exhausted cells. This biological faculty of highly differentiated epithelial cells to maintain their longevity and usefulness by cyclic returns (when functionally exhausted) to their primitive afunctional state, with consequent differentiation and full recovery of their functional efficiency, is offered as an explanation of the functional endurance of these cells in the absence of signs of replacement of worn out elements by means of regeneration.

In studying the effect of a vitamin A deficiency diet the following was observed: in addition to cellular infiltration and congestion along the entire alimentary tract, there is noted an increase in the number of argentaffin cells deriving from mucous and parietal cells. In addition to this interference with the cycle of rejuvenation, avitaminosis causes hydropic degeneration of the parietal cells, signs of which appear every 8th day.

The changes observed indicate that avitaminosis shortens the span of functional life and decreases the functional endurance of the mucous and parietal cells of the alimentary tract. It forces these cells, too, into rejuvenation much sooner than is observed in animals fed a full vitamin diet, and interferes with normal rejuvenation of the parietal cells, causing their degeneration and terminating in atrophy of these most important constituents of the gastric mucosa. Vitamin A is essential for maintaining functional endurance and for a normal rejuvenation cycle of highly differentiated epithelial cells.

HISTOPATHOLOGICAL STUDY OF A FATAL CASE OF HYPERVITAMINOSIS D. Stuart W. Lippincott (by invitation), Montreal, Canada.

Abstract. A 10 months old infant received 40 to 65 times the proper dosage of vitamin D daily for 8 months. This provoked a response quite like that observed in the experimental animal. Histologically this consisted of diffuse cellular degeneration followed by dystrophic calcification. The most advanced lesion occurred in the heart, followed by lesions in the kidney, lung, stomach and small arteries.

Discussion

(Dr. A. B. Wadsworth, Albany, N. Y.) I should like to ask if there was any change in the coagulation of the blood.

(Dr. Lippincott.) Unfortunately this infant died outside the hospital. His

twin brother was in the hospital with similar mild symptomatology. The parents did not permit an autopsy, but after the other infant died, during the course of a cold the twin was brought to the hospital. Blood studies were not made on this infant but the symptomatology when traced back later was quite the same as that of his brother.

(Dr. C. V. Weller, Ann Arbor, Mich.) I am reminded by this case of an experience I had a number of years ago during the first wave of apprehension over the possible dangers of overdosage with vitamin D. I was asked to examine the tissues of an infant who had died at the age of 4 weeks. We found typical myocardial infarction with a calcifying obliteration of the coronary arteries. The history I obtained later was that another child in the family had died in precisely the same manner, but there was no autopsy in the first instance. I felt very confident that death was due to the excessive use of vitamin D. Correspondence with the physicians in charge and their assiduous investigation in the family showed that no known use of vitamin D existed in that family; the circumstances of the family were such as practically to preclude the possibility that any vitamin D product had been administered. I tell this not to throw any doubt on Dr. Lippincott's case, but simply to call attention to the fact that it is not safe to make the *post hoc, ergo propter hoc* assumption. That two members of the family were concerned in each instance establishes a parallelism which may be significant.

THE EFFECT OF VITAMIN D ON THE LIVER LOBULE. Walter Heymann (by invitation) and William B. Wartman, Cleveland, Ohio.

Abstract. It has been found that in young rabbits an increase in multinucleated cells in the peripheral zone of the liver lobule occurs after the administration of viosterol, inactivated viosterol, drisdol, inactivated drisdol and propylene glycol. Young rats and dogs do not show this phenomenon, although vitamin D is known to be just as effective in these animals as in rabbits.

There was no detectable relationship between the effect of vitamin D on the phosphorus and calcium content of the blood and the increase of multinucleated cells in the liver. It is unlikely that an increase in basal metabolism is responsible for the phenomenon since the administration of thyroxin does not lead to an increase in the number of multinucleated cells. Wesson oil and sesame oil are without effect on the liver cells.

The observation that propylene glycol also causes an increase in the number of multinucleated cells suggests that alcohols may be responsible for the reaction. This assumption, however, has yet to be established experimentally.

Discussion

(Dr. Fritz Levy, Elkins, West Va.) I should like to ask Dr. Wartman if he has seen mitotic figures in these liver cells and any larger nuclei than the average nucleus of the liver cell. It is a question whether such binucleated liver cells, which are found in many instances, are due to suppressed cell division after mitotic division of the nucleus, or to amitosis, or finally to a mere fragmentation of the nucleus. There was a paper published last year by Wilhelm Pfuhl, Frankfort on Main (Germany), on binucleated liver cells. He denies that amitosis occurs and described pluripolar mitoses in bivalent cells.

(Dr. N. W. Popoff, Rochester, N. Y.) It was noted in my control studies of epithelial rejuvenation that the liver also shows argentaffin cells and that these cells are readily demonstrated with my method No. I of silver reduction.

I should like to inquire if any effect of vitamin D on these peculiar cells, designated in histology as dark cells, has been noted by investigators.

(Dr. Wartman, closing.) In answer to Dr. Levy's question, I neglected to mention that no increase in the number of mitotic figures was observed. This was shown also by Szittoy. The theory is that the binucleated cells represent amitotic division of the liver cells. No other nuclear abnormalities were noted.

We discussed this problem with Dr. Karsner, who suggested using colchicine at various stages of the experiments, and this will be tried out.

In regard to the argentaffin cells, we have not stained any sections to demonstrate them, so I cannot answer that question; our sections were stained with hematoxylin and eosin.

MYOCARDIAL LESIONS DUE TO COMBINED SALT AND VITAMIN DEFICIENCIES. R. M. Thomas, E. Mylon (by invitation), and M. C. Winternitz, New Haven, Conn.

Abstract. A report by Schrader, Prickett and Salmon in 1937 concerning the effects of a low potassium diet on the tissues of young rats led us to investigate this problem with special reference to the myocardial lesions. It was found that necrosis of the heart muscle fibers occurred in young rats when the diet was deficient in potassium, and also in certain parts of the vitamin B complex. The addition of 10 mg. of potassium chloride to the daily diet was sufficient to prevent the development of the lesions, and conversely the addition of adequate vitamin B to the diet in the absence of potassium was also able to prevent the development of heart lesions. Young hogs when placed on a similar regimen succumbed within 30 to 60 days, and showed extensive heart lesions. Electrocardiographic tracings taken weekly indicated a progressive myocardial damage, characterized by changes in T wave direction and marked increase in conduction times. Groups of young rats were then placed on diets containing various combinations of vitamin and salt mixtures, employing thiamin chloride, riboflavin, and B_6 hydrochloride. By leaving out one of the three vitamins and supplying the remaining two factors, it was found that of the six groups, only the rats that were deficient in vitamin B_6 and in potassium developed myocardial lesions. As before, the addition of potassium chloride to the diet prevented the development of heart lesions without regard to the nature of the vitamin supplements.

VITAMIN A DEFICIENCY AND LESIONS OF THE NERVOUS SYSTEM. S. B. Wolbach and (by invitation) O. A. Bessey and R. L. Swank, Boston, Mass.

Abstract. This study was undertaken in an attempt to clear up the conflicting results concerning the effects of vitamin A deficiency on the nervous system. The effects of long continued malnutrition on peripheral nerves have been discussed. In rats the production of nerve degeneration in vitamin A deficiency depends on the age of the animal. Degeneration of the peripheral nerves and tract degeneration in the spinal cord having a constant pattern are produced if the deficiency is initiated early enough in life. After myelination is complete apparently the deficiency is not productive of nerve degeneration.

THE NERVOUS SYSTEM IN B_1 AVITAMINOSIS. H. M. Zimmerman, New Haven, Conn.

Abstract. The polyneuritides which occur in human beriberi, chronic alcoholism, vomiting of pregnancy, diabetes mellitus, and a variety of other organic diseases, have been shown to result from B_1 avitaminosis (thiamin deficiency). The peripheral nerve lesions are non-inflammatory, consisting first of medullary sheath destruction and later of axone disintegration. Sensory nerves are affected more frequently than motor; the degeneration starts distally and progresses centrally to involve the posterior nerve roots of the spinal cord. In chronic deficiency of this vitamin the posterior spinal columns become demyelinated as in tabes dorsalis.

These lesions have been reproduced in pigeons, rats and dogs with diets deficient only in vitamin B_1. It has been found that in these animals chronic deficiency of thiamin results in hemorrhagic and vascular, astrocytic and microglial proliferative reactions in the gray matter surrounding the third ventricle, the aqueduct of Sylvius, in the floor of the fourth ventricle and in the mammillary bodies. The experimentally produced lesions of this variety resemble closely the changes of chronic polioencephalitis hemorrhagica described by Wernicke in chronic alcoholics. Such lesions are also seen in non-alcoholic patients with chronic vitamin B_1 deficiency.

THE EXPERIMENTAL REPRODUCTION OF WERNICKE'S HEMORRHAGIC ALCOHOLIC ENCEPHALITIS BY B_1 AVITAMINOSIS WITHOUT ALCOHOLISM. Leo Alexander, Boston, Mass.

Abstract. Wernicke's hemorrhagic polioencephalitis occurring in chronic alcoholism in man and a disease produced experimentally in vitamin B_1 deficient pigeons in the presence of ample supplies of other vitamins are identical in their topographical distribution and in their morphological and histological characteristics.

Wernicke's hemorrhagic polioencephalitis can be produced with significant regularity as a complication of beriberi, if the vitamin B_1 deficient pigeons are given large supplies of other vitamins (A, B_2, C, D).

If the pigeons are fed an entirely vitamin-free diet, the resulting beriberi will only rarely be complicated by lesions of the Wernicke type.

Wernicke's disease cannot be produced in pigeons receiving crystalline vitamin B_1 (thiamin), although they may be deficient in all other vitamins or of any one other vitamin for a period of over 6 months.

These observations suggest that vitamin B_1 possesses antiangiodegenerative properties in addition to its antineuritic properties, and that smaller amounts of vitamin B_1 are sufficient to act as an antiangiodegenerative agent than are sufficient to act as an antineuritic agent.

The administration of high doses of vitamin A, B_2, C or D in vitamin B_1 deficiency probably raises the vitamin B_1 requirement of the tissues, and angiodegeneration manifests itself soon after the onset of the neuronal degeneration.

Discussion of papers by Dr. Zimmerman and Dr. Alexander

(Dr. Robert G. Green, Minneapolis, Minn.) I should like to mention that there is a condition apparently identical with the one just described which occurs in fox and mink farms throughout the country in fairly large out-

breaks, sometimes almost decimating a fox farm. The lesions in adult foxes are identical with those described for Wernicke's disease. The disease occurs when the diet contains about 15 per cent of fresh fish. It was first described in our Minnesota Wildlife Disease Investigations, and we have called it Chastek paralysis, or fish disease. I saw one outbreak on a ranch where only 5 per cent of the diet was fish, but the diet also contained 2.5 per cent of cod liver oil. We have followed this condition for a number of years, trying to determine what the outbreaks were due to. Within the past year we have stopped outbreaks and cured individual animals with thiamin, although it would seem that there are other mild deficiencies present in addition to the B_1 deficiency.

(Dr. S. B. Wolbach, Boston, Mass.) I think someone should say something for the peace of mind of the audience. I am certain that you must be a little confused. I have been very much confused in the past. I do not know that I am clear now. I know that Dr. Zimmerman has gone farther than we have. He has observed things we have not observed in Boston. The only essential difference in our work and his is his statement that myelin sheath changes precede changes in the axis cylinder. At present, at least, we think the reverse to be true, but we have been wrong so often that we may be wrong again. There are many factors involved in vitamin research, particularly where one attempts to correlate deficiencies with morphological changes. I should say our group in Boston has become resigned to disappointment, to the discarding of many months and even years of work, and we have become exceedingly tolerant of any careful work done by others, so that I think we will have to be very patient, but very hopeful, because I think these papers this afternoon, particularly those of Drs. Zimmerman, Alexander, and Wartman and Heymann, all represent very important advances.

(Dr. Zimmerman, closing.) I want to thank Dr. Wolbach for his great kindness in mentioning the work we have done in his paper. I do want to lay the ghost of the question whether starvation produces changes in the peripheral nerves, and say what I neglected to say before, which is that we have never performed an experiment on deficiency diseases in the last 2 years without utilizing an inanition control for each of the animals we have fed the experimental diet, that is, the control animal received only that caloric intake which the experimental animal received the day before. No peripheral nerve demyelination ever developed in the inanition controls.

I think the myelin sheath degeneration which Dr. Wolbach has mentioned precedes the axis cylinder degeneration, partly because it is more plausible, and partly from our histological evidence. It is well known that the early deficiency of B_1, which has dramatic clinical symptoms, can be cured almost miraculously within a few hours by the injection of thiamin, and with axis cylinder degeneration that would hardly be possible. Furthermore, I know of no instance of degenerative disease in man where axis cylinder degeneration precedes myelin sheath degeneration.

(Dr. Alexander, closing.) I am fascinated by Dr. Green's statements about the fish disease of foxes. I should like to have a talk with him later about this. I hope his studies will find their way into the literature. It is interesting that once more a fish diet looms importantly in the pathogenesis of a vitamin deficiency. The most clear-cut pure cases of beriberi in man are those observed among the Japanese fishermen who live on a diet consisting exclusively

of rice and fish. I had a talk with Dr. Shimazono of Tokyo about this, and he told me that these rather well nourished fishermen show pure vitamin B_1 deficiency, which is never complicated with other deficiencies. This is due to the fact that fish meat and fish liver contain an abundance of vitamins A, D, and B_2, while completely lacking in vitamin B_1. Thereby, a fish diet reproduces the situation existing in those of my experimental animals which were vitamin B_1 deficient, but which were receiving a large supply of other vitamins. It is an interesting confirmation of our work, therefore, to hear from Dr. Green that a fish diet produces Wernicke's disease in foxes, just as the regimen of vitamin B_1 deficiency and abundance of other vitamins produces Wernicke's disease in pigeons. The situation is similar in chronic alcoholics. They are deficient in vitamin B_1 but receive abundant supplies of vitamins A, D and riboflavin from spiced meats, fat sausages, liverwurst, and so on. Correspondingly, chronic alcoholic patients rarely develop riboflavin deficiency or anemia.

AN EXPERIMENTAL STUDY OF THE NEUROPATHOLOGICAL ASPECTS OF RIBOFLAVIN AND VITAMIN B_6 DEFICIENCIES IN DOGS. Clarence M. Lightner (by invitation) and Wiley D. Forbus, Durham, N. C.

Abstract. The work here reported concerns the pathological, anatomical and physiological effects produced in dogs by diets deficient specifically in (*a*) riboflavin and (*b*) the B_6 component of the B_2 complex. Adult dogs and young puppies were divided into 5 groups and fed as follows:

GROUP A. *B_2 Complex Deficient:* Three adult dogs and 2 puppies were fed a basal diet consisting of:

Casein (vitamin-free, Harris)	30%
Sucrose	36%
Bone ash	4%
Vegetable lard	27%
Salt mixture (Osborne-Mendel, including manganese and copper)	3%

and supplemented with

Crystalline vitamin B_1	0.4 gm. daily
Nicotinic acid	1.0 mg. per kg. daily
Vitamin A (cod liver oil)	1750 units per kg. daily
Vitamin D (cod liver oil)	175 units per kg. daily

GROUP B. *B_6 Deficient:* Five adult dogs and 3 puppies were fed a basal diet with supplement as in Group A, to which was added daily 0.1 mg. per kg. of crystalline riboflavin in a 0.025 per cent aqueous solution.

GROUP C. *Riboflavin Deficient:* Four adult dogs and 1 puppy were fed a basal diet supplemented as in Group A, the sucrose in the basal diet being replaced by molasses, an adequate source of B_6.

GROUP D. *Dietary Control:* Three adult dogs and 1 puppy were fed a basal diet supplemented as in Group A. To this was added crystalline riboflavin as in Group B, and B_6, supplied by substituting molasses for the sucrose as in Group C.

GROUP E. *Normal Control:* Two adult dogs and 1 puppy were fed a stock laboratory diet.

The animals were fed the diets for a maximum period of 302 days. The

nervous system was studied grossly and by discriminative histological methods after complete autopsy.

Results: 1. In none of the animals, experimental or control, were we able to demonstrate significant degenerative or other pathological changes in the central or peripheral nervous system.

2. The clinical course of B_6 deficient dogs was characterized by progressive anorexia and loss of weight and a suggestive anemia. The clinical course of the riboflavin-deficient animals was characterized by recurrent attacks of weakness and complete collapse described as typical of riboflavin deficiency by Cowgill and associates, as well as Sebrell and others.

3. In view of the failure of this experiment to confirm the work of Zimmerman and Burack, and of Zimmerman, Cowgill and Fox, one of the following possibilities is suggested: (*a*) deficiency of riboflavin or B_6 alone or in combination does not give rise to degenerative changes in the nervous system; (*b*) while complete deficiency in riboflavin and/or B_6 does not give rise to degenerative change in the nervous system, prolonged moderate deficiency of these factors may result in this change; and (*c*) deficiency of some component of the B_2 complex other than riboflavin or B_6 may account for degenerative changes in nervous tissue.

Discussion

(Dr. Maxwell M. Wintrobe, Baltimore, Md.) I should like to ask Dr. Forbus if his animals were well nourished in general, and also whether provision was made in the diet for those portions of the "B complex" group other than B_1 and nicotinic acid.

(Dr. H. M. Zimmerman, New Haven, Conn.) I had occasion some 2 or 3 weeks ago to read the paper which Dr. Forbus prepared for publication and I feel it substantiates the conclusions which he presented this afternoon. That paper describes certain findings which we have been able to confirm, and which we find to be true for riboflavin deficiency. I think if the Marchi method and perhaps also Sudan III stains were used on the spinal cord in riboflavin deficiency, one could demonstrate even more extensive lesions in the fiber tracts than in B_1 deficiency. There are no changes in the brain itself.

(Dr. Leo Alexander, Boston, Mass.) I should like to ask Dr. Forbus whether he observed anything comparable to the lesions which Sebrell described in man.

(Dr. S. B. Wolbach, Boston, Mass.) I wonder whether these riboflavin deficiencies are accompanied with any clinical neurological disturbances, because we have run riboflavin-deficient rats, many of them to death, and have never seen signs of neurological lesions.

(Dr. H. M. Zimmerman, New Haven, Conn.) In rats, we too have failed to obtain any lesions, and that is true because our rats seemed to die off long before they developed anything in their nervous systems. With riboflavin-deficient dogs we obtained, after 400 days, quite marked ataxic changes which were more striking than in the B_1 deficient animals.

(Dr. Forbus, closing.) Dr. Wintrobe has asked about the state of nutrition of the animals. The B_6 animals did not maintain a good state of nutrition throughout the period. They showed anorexia and loss of weight and certain of the animals died after losing about half of their original weight. The animals which were placed on the basic diet plus the riboflavin and B_6 supplement maintained their weights in reasonably normal limits. The riboflavin

dogs lost some weight. As for the significance of the components of the B_2 complex other than B_6 and riboflavin, we can say nothing. Our experiment was not designed to study the influence of these factors upon the animal. We did do certain preliminary studies with the "filtrate factor," but I have nothing to report since the experiments were not critical. We restricted our work entirely to the two factors which we thought we could control accurately.

I should like to have said more about histological methods employed, a matter to which Dr. Zimmerman has referred. We did not use the Marchi methods for the detection of changes in either the brain and cord or peripheral nerves. Scharlach R was used on numerous segments of the cord of each animal. It was not employed on the brain or the peripheral nerves since the Weigert technic in combination with several other methods for demonstrating degenerations in both axis cylinders and medullary sheaths showed no significant alterations. In the various segments of the cord which were stained with scharlach R it was possible to gain a satisfactory view of the attached portions of the posterior and anterior nerve roots. In none of the many sections which were studied by this technic was there evidence of degeneration demonstrated by this method.

CEREBROSPINAL PATHOLOGY OF EXPERIMENTAL POLIOMYELITIS IN THE EASTERN COTTON RAT (SIGMODON HISPIDUS HISPIDUS) AND IN THE WHITE MOUSE (MUS MUSCULUS). R. D. Lillie and (by invitation) Charles Armstrong, Washington, D. C.

Abstract. Intracerebral inoculation with the Lansing strain of poliomyelitis virus produces in cotton rats (*Sigmodon hispidus hispidus*) and white mice (*Mus musculus*) an inflammatory process characterized by necrosis of nerve cells, polymorphonuclear leukocyte exudation and invasion of necrotic cells, neuronophagia by macrophages, diffuse and focal cellular gliosis, vascular endotheliosis and infiltration of the sheath with lymphocytes. In the brain the dorsal portion of the pons shows the greatest involvement, then medulla, midbrain, thalamus and cerebellar roof nuclei. The cerebral cortex in both species shows relatively little involvement, most in the hippocampus. The corpora striata and cerebellar cortex show the least involvement. In spite of intracerebral inoculation into the thalamomesencephalic region, the earliest observed lesions are found in the spinal cord in both species.

The process in both species is considered closely similar to the reaction of poliomyelitis in man and in monkeys. In general, the amount of lymphocytic infiltration in the vessel sheaths is less in cotton rats than in mice, monkeys or in man.

Discussion

(Dr. Edward C. Rosenow, Rochester, Minn.) I am very much interested in the lesions produced in these experiments, and I am wondering whether the lesions in the brains of rats and mice, and the symptoms were more encephalitic in these species than they are in the monkey. We have had what appeared as polioencephalitic takes in cotton rats following intracerebral and intraperitoneal inoculation of three different poliomyelitic viruses. The symptoms were more those of encephalitis than of poliomyelitis. The microscopic sections have not yet been examined. We have not had time to pass these strains through other animals. Another interesting point this work

brings up is this: We have inoculated mice with poliomyelitic virus and have obtained flaccid paralysis, but we cannot carry the virus through mice consecutively for more than 2 or 3 passages, and I am wondering whether Drs. Armstrong and Lillie have had success in passing the mouse virus from animal to animal.

(Dr. Lillie.) In reply to Dr. Rosenow's question, I believe in the first place that Dr. Armstrong's success in passing this virus serially through mice for many passages now is due to the fact that that strain of virus has become acclimated to a rodent host before it was put into the mouse. It was passed through the cotton rat from 17 to 18 passages before we tried it in the mouse. We tried it before and had no luck with it. This Lansing strain which was used in the mice was first carried serially through the cotton rat, which is another type of beast.

In regard to the question about flaccid paralysis, cerebral nerve paralyses are rather difficult to observe in small rodents. The things that have been striking are the hind and fore limb paralyses. Some have shown definite jaw paralysis. Dr. Rosenow spoke of the brain lesions which we have shown. When one studies the monkey or man after death, one gets very much the same portions of the brain involved as in the cotton rat and mouse, namely the brain stem, the medulla, midbrain, thalamus and cerebellar roof nuclei. I should stress again that probably while paralysis occurs in 2 or 3 days after inoculation in most cases, and spinal cord lesions are evident in 2 or 3 days after inoculation, at that time if you hunt through the brain, despite the intracerebral route of inoculation, you will not find a sign, except perhaps a needle tract, and you are lucky to get that.

(Dr. Malcolm H. Soule, Ann Arbor, Mich.) How many times has Dr. Armstrong been able to go back to his monkey virus of the Lansing strain and produce infections in cotton rats and white mice and carry it serially?

(Dr. Lillie.) We have not gone back to work on the original monkey virus again to any extent. The current problem has been in the other direction. Dr. Armstrong was trying to get other strains of virus to grow, considering that more important right now than repeating the work. I believe it has been repeated but that is one detail concerning which I am not too competent to talk.

(Dr. Soule.) I think Dr. Rosenow has made a most outstanding contribution if he has carried three separate strains of poliomyelitis virus into cotton rats.

(Dr. Lillie, closing.) I hope he is able to carry these serially and prove it.

TRANSIENT SPASM AND THE CENTRAL NERVOUS SYSTEM (HISTOLOGICAL STUDIES). A. J. Nedzel, Chicago, Ill.

Abstract. It has been noted that vascular spasm as an etiological factor in evoking some clinical entities is more prominent in the northern parts of our country. For instance, the greater number of persons suffering from multiple sclerosis in this region is evident. Later experimental work (Putnam) points to vascular obstruction as the causative factor in this disease. But there is also much clinical evidence that makes it quite probable that either undue transient vessel spasm or undue transient dilatation of the vascular bed is often the cause of some of the so-called functional diseases. The question arises, will these functional vascular episodes, if prolonged or repeated, lead

to the establishment of some permanent lesions in the central nervous system, where a transient vascular spasm is not so readily induced.

A series of experiments were set up in which rabbits and dogs received single and repeated intravenous injections of pitressin (beta hypophamine) which causes transient vascular spasm of the arterioles and of the capillary bed in general, although actually this is less pronounced in the central nervous system. Histological material from the central nervous system, mostly of the spinal cord, has been studied. Definite changes have been observed such as swelling of the endothelium of the capillaries, small hemorrhages, increase in the number of leukocytes, accumulation of glial cells around the blood vessels, appearance of thrombotic blood vessels, and so on. Also, gliosis is observed and swollen myelin sheaths and axons are found in scattered patches. The changes in the nerve cells range from simple hydration to a necrobiosis.

Discussion

(Dr. Kornel L. Terplan, Buffalo, N. Y.) I should like to ask one question. Has Dr. Nedzel examined in his material particularly the cortical layers of the cerebrum? We were frequently impressed by marked and almost selective damage seen especially in the third layer of the cerebral cortex in different areas in some cases, with histories which pointed to spastic events before death. In these cases we could not find similar damage to the nerve cells in the medulla and pons.

(Dr. Nedzel, closing.) The brain, particularly the gray matter, was studied. Changes similar to the ones here reported were found, mostly in the hippocampus major, though not to such an extent as in the spinal cord. This concerns dogs, which are more stable, and more readily overcome the transient spasms evoked by pitressin injections. Rabbits are more responsive. In 2 cases these animals developed a spontaneous general septicemia with marked changes in the brain, although they received only pitressin injections. This is the reason why I report here only findings in dogs, a more stable and less responsive animal.

There is an impression that the lesions artificially evoked by transient spasms (pitressin injections) heal readily. Only if the pitressin injections are repeated for a long time do they lead to the establishment of permanent lesions. It is believed that these "insignificant" minor changes can be definitely connected with some of the functional diseases of a vascular origin. In other words, the repeated vascular episodes (spasms) do not pass without leaving some noticeable trace in the place of their occurrence.

ENDOMETRIAL RESPONSE TO STILBESTROL IN RADIUM MENOPAUSE. R. C. Grauer and (by invitation) C. Beall and G. R. Wilson, Pittsburgh, Pa.

Abstract. Ten patients ranging in age from 42 to 50 years, who had been given 1200 to 1600 mg. hours of radium for bleeding dysfunction, were studied. Endometrial biopsies were done before radium was inserted to rule out malignancy. One to 2 years after radium insertion, vaginal smears and endometrial biopsies were attempted. No biopsies could be obtained. Three milligrams of diethyl stilbestrol were administered orally for 16 days and again biopsies and vaginal smears were taken. Treatment was continued for 5 weeks and endometrial response was then observed for the first time. Vaginal epithelial response also occurred.

Treatment was further continued for 4 months. It was found that a proliferative type of endometrium which tended to duplicate the appearance of the endometrium before radium insertion was secured. The stilbestrol stimulated to proliferation glands, stroma and myometrium. The type of response varied from the physiological menopause cases in that if only a vestige of tissue remained which had not been destroyed by radium, it was stimulated to proliferation, so that contiguous areas of hyalinized collagen and stimulated endometrium and myometrium occurred. The dosage and time element required to stimulate the radium treated cases were twice as great as in the physiological and surgical cases.

Discussion

(Dr. Warren C. Corwin, New Brunswick, N. J.) Dr. Ephraim Schorr and his associates have recently done considerable work on the toxicity of stilbestrol following its clinical use. I do not know whether he has published these results yet or not. I am sure others have done this type of work too. I should like to ask Dr. Grauer if any of his patients showed any signs of toxicity, particularly as regards gastrointestinal upsets.

(Dr. R. C. Grauer.) I avoided going into the clinical side, but we were also interested in that side of the work. I think Schorr and Papanicolou published a preliminary report of their results in the Journal of the American Medical Association. Our experience has been that there was nausea in about 30 to 35 per cent of the cases the first week or two of treatment, and vomiting in a smaller percentage of cases. This occurs, I think, where the initial doses are large. The radium cases received 1 mg. three times a day. When we started on the physiological and surgical cases we began with 0.1 mg. twice a day and increased it gradually. When this was done, we did not experience any toxic effect in so far as the gastrointestinal tract was concerned. We had only 1 case which showed an erythematous eruption of the skin. Schorr and Papanicolou mentioned several. One thing we observed which they did not mention is that there was transient knotting of the skeletal muscles, particularly when the patient got out of bed in the morning. When treatment is continued all these symptoms disappear. It is now approximately 5½ months since treatment was instituted and the patients show no untoward symptoms.

(Dr. Corwin.) Were there any psychiatric manifestations?

(Dr. Grauer.) There were none in our series.

THE RELATIONSHIP OF CERTAIN OF THE ENDOCRINE GLANDS TO CHANGES IN THE FAT CONTENT OF THE LIVER. Warren C. Corwin, New Brunswick, N. J.

Abstract. During the past 4 years several investigators (notably Best and Campbell) have demonstrated that alkaline extracts of the anterior lobe of the pituitary are capable of increasing the fatty acid content of the liver. McKay and Barnes found that such an increase could not be obtained in previously adrenalectomized animals. These findings, as well as supplementary observations, led workers in the field to believe that fatty changes in the liver are directly under the control of the adrenal glands, but there has not as yet been forthcoming any very tangible proof.

Experimenting with rats I have been able to corroborate the work of Best and Campbell, and of McKay and Barnes, and have further shown that not

only various alkaline extracts but also crude saline extracts, as well as a purified adrenotropic fraction of the anterior lobe of the pituitary, markedly increase the fat content of the liver in a period of 24 hours. I have not, however, been able to demonstrate any appreciable increase following the injection of varying amounts of adrenal cortical extract.

As has been shown by other investigators, adrenalin also increases the fat content of the liver. It is very tempting, therefore, to asssume that the effect of pituitary extracts on liver fats is mediated through the medulla. However, a good many of my endocrine-minded colleagues object to postulating a medullotropic hormone. I have already planned experiments on adrenal-demedullated animals to shed further light on this question, if possible.

In addition, I have found that thyroidectomy is quite as effective as adrenalectomy in preventing an increase in the fat content of the liver following injection of anterior pituitary extracts. A single preliminary experiment indicates that thyroxin may have a lipotrophic effect. To date I have been unable to obtain for testing a thyrotropic fraction of the anterior pituitary which is free of adrenotropic activity.

In conclusion, it appears that the hepatic lipotrophic effect of extracts of the anterior lobe of the hypophysis is mediated through the thyroid and adrenal glands. Nothing more definite than this can be said at the present time.

TRANSITORY INVASION OF THE BLOOD STREAM DURING LABOR BY ANAEROBIC NON-HEMOLYTIC STREPTOCOCCI. G. M. Mackenzie and (by invitation) Helen Fitzgerald, Cooperstown, N. Y.

Abstract. In a series of 205 patients cultures were taken from the cervix or upper vagina at the beginning of labor. Blood cultures were taken immediately after delivery. Both vaginal and blood cultures were incubated aerobically and anaerobically. Anaerobic non-hemolytic streptococci were the most common microorganisms isolated from the birth canal. *Staphylococcus albus*, diphtheroids and aerobic strains of *Streptococcus anhemolyticus* were also frequently isolated. *Streptococcus hemolyticus* was obtained only twice. Eleven of the patients were shown to have transitory bacteriemia during labor. Anaerobic strains of non-hemolytic streptococcus were isolated from the blood cultures of 8 patients. From the blood cultures of 3 patients facultative anaerobic non-hemolytic streptococci were isolated. In 9 of the 11 patients with bacteriemia cultures from the birth canal yielded the same type of streptococcus as the blood culture. Ten of the 11 patients with bacteriemia had no clinical manifestations of infection. From these observations it appears that transitory invasion of the blood stream without symptoms of infection occurs not infrequently during labor.

THE BACTERIOLOGY OF POSTMORTEM FEBRILE CONDITIONS. George R. Lacy and Mortimer Cohen, Pittsburgh, Pa.

Abstract. The work presented covers a period of 11 years. During this time studies were made on 34,538 patients who were admitted to the obstetrical service at the Elizabeth Steel Magee Hospital, Pittsburgh. Maternal deaths due to all causes numbered 315, or a mortality rate of 9.1 per 1000 admissions. Admissions to the same obstetrical service for the year 1939 amounted to

3531 and the number of deaths 17. The mortality rate for the one year was 4.8 per 1000 admissions.

The causes of death among the 315 who died were classified as puerperal sepsis 18.7 per cent, abortions with sepsis 17.5 per cent, toxemias including eclampsia 19.4 per cent, hemorrhage and shock 14.9 per cent, and all other causes 29.5 per cent.

Intrauterine swabs were secured from all patients who developed a temperature of 101° F. during the puerperium. Positive cultures were obtained from 460 patients. The clinical diagnoses of conditions presented by these 460 patients were: puerperal sepsis 38 per cent, abortion with infection 18.9 per cent, abortion with mild fever 18.7 per cent, lochiometra 18.5 per cent, and retained placental tissue 5.9 per cent.

The important organisms isolated were hemolytic streptococci, non-hemolytic streptococci, *B. coli*, *Staphylococcus aureus*, and *Clostridium welchii*. Hemolytic streptococci were seen more frequently than all other organisms combined and were more highly pathogenic, as shown by the fact that most of the deaths were due to hemolytic streptococci.

Direct smears and cultures of materials from uteri were found to agree in practically all cases, provided swabs containing the inoculum were left in the tubes of broth medium during incubation of the cultures. Micro-aerophilic streptococci were usually induced to grow on aerobic blood agar plates after three or four generations in broth tubes with swabs.

Discussion of papers by Drs. Mackenzie and Fitzgerald, and Lacy and Cohen

(Dr. Paul R. Cannon, Chicago, Ill.) I should like to ask Dr. Mackenzie if he has made any observations on the presence of immune bodies to these anaerobic non-hemolytic streptococci. I assume there were no residual infections, and I wonder if these patients who were already infected may have had an increased ability to eliminate the organisms from the blood stream.

(Dr. B. J. Clawson, Minneapolis, Minn.) I should like to ask Dr. Lacy whether the abortions were spontaneous or induced.

(Dr. Mackenzie.) I should like to ask Dr. Lacy if, in those patients who had fever and clinical evidence of infection but whose blood cultures showed nothing, their blood cultures were incubated anaerobically.

In answer to Dr. Cannon's question, we did not make any study of immune bodies. These patients were uninfected; they were normal labor cases. We took blood cultures subsequently on a number of the cases and they were all sterile.

(Dr. Lacy.) In answer to Dr. Clawson's question, some of the cases that were included as abortions were self-induced, some of them were therapeutic abortions, and some of them were spontaneous, so that the ones I discussed in particular are those which showed infection from any cause.

In reply to Dr. Mackenzie's question, I presume he means did we place the cultures in an anaerobic jar. In most cases we did not. The methods which we used were anaerobic only to the extent of the use of sterile swabs transferred to broth tubes as we described. We found that practically all of the ordinary anaerobic bacteria would grow readily by this method. We did not put them in anaerobic jars. In all cases where it was thought probable that streptococci should be found but were not, transfers were made to blood agar slants. These tubes were then heated, above the medium, in the direct flame, and quickly closed with a rubber stopper. This gave a sufficient reduction in

oxygen tension to permit growth of the organisms occasionally. A few transfers of this type were usually sufficient to cause the organism to grow aerobically.

STUDIES ON BACILLEMIA IN EXPERIMENTAL TUBERCULOSIS. John S. Howe, Chicago, Ill.

Abstract. This report summarizes work carried on over a period of 5 years on the occurrence of bacillemia in dogs infected experimentally with tuberculosis, from which the blood was cultured daily for periods of 6 to 12 weeks.

Twenty-six dogs in all were used, of which 23 were injected intravenously with a suspension of large doses of virulent human type tubercle bacilli (H_{37}) in mineral oil, kaolin and saline, and 3 were injected subcutaneously into the chest wall with a similar suspension.

Blood was drawn daily from the femoral artery and injected into guinea pigs. All guinea pigs were sacrificed 3 months after inoculation and autopsied to establish the presence or absence of tuberculous lesions of the injection type.

Charts showing the daily distribution of bacillemia in dogs observed over the same period were analyzed in relation to various factors, including, in two series, the pH of the dogs' arterial blood.

Twenty-three of 26 dogs injected intravenously and 2 of 3 dogs injected subcutaneously showed sporadic bacillemia on at least one occasion, as demonstrated by guinea pig inoculation. Dogs observed over the same periods of time showed a definite tendency to have roughly simultaneous periods of bacillemia. When these periods of bacillemia were compared with the curve for the pH of the dogs' blood, the tendency was quite definite for bacillemia to occur with a relatively low pH level. The possible explanation of these results is discussed briefly.

Discussion

(Dr. Esmond R. Long, Philadelphia, Pa.) We are interested in bacillemia in tuberculosis, especially in cases where the infection is small and bacilli in the blood must be very few. It was interesting in Dr. Howe's experiments that the concentration and frequency with which the bacilli appeared in the blood were high. There is the Duchaine method of concentration with which Dr. Howe is probably familiar, which I might describe here. In animals that had slight infections Duchaine produced sterile injuries in the pleural cavity and noted that the bacilli, which could not be obtained in the blood stream, tended to accumulate in such a number that he could make cultures from the fluid in the artificial inflammation produced. That is a point which probably has some bearing on the metastatic lesions, such as lesions in the bones, which sometimes develop after trauma in cases of human tuberculosis.

(Dr. Arthur J. Vorwald, Saranac Lake, N. Y.) I should like to ask Dr. Howe if there is any difference in the character and distribution of the lesions in those animals that showed no bacillemia, as compared with those that did.

(Dr. Howe, closing.) In answer to Dr. Vorwald, the correlation between bacillemia and the distribution of the lesions was much less striking than I had expected. The dogs that showed very heavy bacillemia in some instances died of progressive tuberculosis and showed what one might consider a miliary tuberculosis. However, that was only in 1 or 2 dogs. By far the largest

number of the dogs lived during the entire period of observation and at autopsy, even when they had shown heavy bacillemia, the lesions were largely in the lung.

In answer to Dr. Long, I am familiar with Duchaine's method. My particular reason for using a direct blood culture in this experiment was that I thought it was not subject to some of the criticisms that had been made of Duchaine's method.

THE SIGNIFICANCE OF BRONCHIAL OBSTRUCTION IN PULMONARY TUBERCULOSIS IN CHILDREN AND ITS RELATION TO EPITUBERCULOSIS. Kornel L. Terplan, Buffalo, N. Y.

Abstract. The close interdependence of occlusion of the bronchial tubes by tuberculous granulation tissue and lobar or lobular atelectasis in the course of primary tuberculosis in children was demonstrated by postmortem examination in 5 cases. These endobronchial tuberculous lesions with complete atelectasis of corresponding lobules were found in recent stages as well as in the form of pencil-like, calcific structures obturating the lumens of third order bronchi. Some of these latter represented healed states of the Ghon focus or additional calcified and ossified endobronchial lesions, in close relation to the primary focus, apparently following early intrabronchial spread. In these later cases the corresponding lobules showed the typical picture of old collapse induration with funnel-like retraction of the pleural surface. In another case which was clinically diagnosed as epituberculous pneumonia of the right upper lobe and which had been observed over a period of several years with a series of X-rays until shortly before death, extreme collapse induration was found postmortem, with unusually marked shrinkage of the entire right upper lobe due to calcific occlusion of its main bronchus. In an additional clinical observation of a typical epituberculous pneumonic shadow in a child 3 years of age, soft foreign particles were expectorated from the bronchus leading to the involved lung during bronchoscopic examination. These, microscopically, were found to be caseated lymph node structures containing tubercle bacilli. Since then the massive shadow has markedly cleared up and the child's subjective condition returned to normal within a few days.

The anatomical findings here presented do not support the view that epituberculosis is a true tuberculous pneumonia which heals by resolution or organization. They rather confirm in part the view already stressed by Roessle that the anatomical substrate of epituberculous pneumonia is atelectasis. In our series, however, this atelectasis was always caused by obturation of bronchial lumens with specific tuberculous structures.

THE PRESENCE OF VIABLE TUBERCLE BACILLI IN LESIONS OF THE GHON COMPLEX OF CHILDREN. William H. Feldman and (by invitation) Henry F. Helmholz, Rochester, Minn.

Abstract. This is a preliminary report of a study to determine the presence of virulent tubercle bacilli in the lesions of the primary complex of pulmonary tuberculosis of children aged up to 15 years. Fresh specimens were received from hospitals in four different metropolitan areas. From most of the lesions a portion was preserved for histological section, and the balance was used to inoculate guinea pigs. From a total of 20 cases virulent tubercle bacilli were

demonstrated in 4. The results of the study to date indicate that the majority of the lesions of primary tuberculous infection in children become sterile during the first few years of life. A more detailed report is contemplated after a larger series of cases has been studied. Additional material is solicited.

Discussion of papers by Drs. Terplan, and Feldman and Helmholz

(Dr. Howard T. Karsner, Cleveland, Ohio.) I hope it may be permissible to raise some questions about both of these papers on tuberculosis. The term "epituberculosis" and the term "Ghon lesion" were used freely. I should like to ask if Dr. Terplan will explain what is meant by epituberculosis, and if Dr. Feldman will explain what is meant by the term "Ghon lesion."

(Dr. Esmond R. Long, Philadelphia, Pa.) I believe there is a correlation between this work of Dr. Feldman's and certain epidemiological facts now becoming well known. Formerly when tuberculous infection was far more common than it is now, practically all children and adults with calcified lesions that could be seen on X-ray examination reacted to tuberculin. In recent years hundreds of examples of what appear to be calcified lesions on X-ray examination have been found in people who are not positive to tuberculin. For a time this raised a good deal of question as to the validity of the tuberculin test. Recently at the Henry Phipps Institute in Philadelphia we have seen many children who over a period of years were positive to tuberculin become negative, including a number who originally had active lesions which ultimately passed into the stage that Dr. Feldman has described. I think the opinion is growing that when the tuberculin reaction is positive in a child or an adult with a calcified lesion, it is not necessarily positive because of that lesion. The sensitiveness that is detected in such cases is probably often the result, not of the lesion seen as a calcified nodule, but of a later infection.

(Dr. Norbert Enzer, Milwaukee, Wis.) I should like to ask if there is any information as to the multiplicity of the primary lesions.

(Dr. Terplan.) The term epituberculosis or epituberculous pneumonia has been known for about 20 years. It was introduced by Eliasberg and Neuland. Since that time, and especially in later years, there has been a quite active discussion of the nature of this disease, especially in journals of tuberculosis and childhood diseases. But the term is also mentioned in textbooks of pathology. This terminology was used by Eliasberg and Neuland to designate clinically most impressive and massive "infiltrations" of the lung, especially in the upper fields, which were seen in infants and children known to have a tuberculous infection. Clinically they showed amazingly mild constitutional symptoms in spite of this extensive area of apparent pulmonary consolidation. The condition frequently was discovered incidentally by X-ray. As these massive shadows showed a rather remarkable spontaneous regression, it was thought that they represent either non-specific pneumonic changes in close relation to the primary focus, or specific tuberculous bronchopneumonia. It was thought that this so-called tuberculous pneumonia heals by resolution or organization. This latter view is still, in spite of the increasing proof to the contrary, the leading viewpoint among pediatricians. The work of Roessle has shown that the anatomical basis in most cases of epituberculosis is really atelectasis. He felt that this atelectasis in almost all of his cases was due to compression of a bronchus by an enlarged lymph node in the early caseated phase. The work I tried to present before this Society includes only cases of

obturation atelectasis, all of which were brought about by complete obstruction of the bronchus from endobronchial tuberculosis. This, I feel, answers the question of Dr. Karsner.

(Dr. Karsner.) What I want to know is, what is the lesion?

(Dr. Terplan.) The lesion is massive atelectasis following complete obstruction of bronchi and bronchioles by tuberculous structures. We have proved, especially with the last case, that occlusion of the bronchus by tuberculous granulation tissue has caused the massive X-ray shadows diagnosed as epituberculous pneumonia. The clinical evidence that these massive shadows might gradually clear up, the children being practically healthy all the time, is in favor of this view. The shadow might disappear almost completely, or only a narrow band might remain, caused by the ultimate collapse induration.

(Dr. Feldman.) Dr. Karnser's question concerning the Ghon tubercle is, I believe, an academic question and requires an academic answer, so I will ask Dr. Long, who is an expert, to give it.

In answer to Dr. Enzer's question in regard to the multiplicity of lesions, we have not received all the information on the autopsy protocols; consequently we do not have the information Dr. Enzer requests.

(Dr. Long.) Anton Ghon of Prague and Vienna wrote a great deal about the primary complex of tuberculosis. He always gave credit to several predecessors who published descriptions of the particular lesion concerned, which is a nodule in the lung, and a secondary nodule in a lymph node going on to calcification — the primary complex of Ranke. Because Ghon wrote so well on the subject many people have attached his name to the healed lesion of primary tuberculous infection. He himself would not claim credit for it.

The President called on Dr. Karsner for further discussion

(Dr. Karsner.) The reason for these questions was not, as might have been assumed, mere pedantry, but I do think that in the presentation of scientific material when terms are used there should be some clear expression of what those terms mean. My conception of the so-called Ghon lesion, meaning by that the lesion in the lung rather than the entire complex, is exactly as Dr. Long has expressed it.

Dr. Terplan has spoken of the lesion of epituberculosis being principally one of atelectasis or collapse of the lung, distal to areas of obstruction of the bronchi. I would raise another question about that. Dr. Reichle has studied the matter and there may be some ground for thinking that these shadows in the X-rays may be due to a bronchopneumonia, which morphologically is like that of ordinary bronchopneumonia, but special staining shows that the pneumonic region is filled with tubercle bacilli. Naturally we cannot get any exact information about the resolution of such a lesion, but certainly in the course of the disease in children shadows with hazy outlines appear in the X-ray field, which completely disappear, and examination at autopsy in those same fields shows no residuum of the original lesion. If the original lesion had been atelectasis, that ought to be permanent or leave some trace. On the other hand, it is conceivable that as other bronchopneumonias resolve, so may these bronchopneumonias which are rich in tubercle bacilli also resolve. My reason for asking the question was not pedantic but to find out what you mean by this term epituberculosis. Evidently we are at some disagreement as to whether it means atelectasis or a type of bronchopneumonia so rich in tubercle bacilli as to be due probably to tubercle bacilli.

STUDIES ON EXPERIMENTAL ALLERGIC INFLAMMATION IN THE LIVER. George Hartley, Jr., and (by invitation) Clarence C. Lushbaugh, Chicago, Ill.

Abstract. These investigations were undertaken to study allergic inflammation in a parenchymatous organ and to compare the nature of the response in the liver with that which occurs in the skin.

One cc. of 3 per cent crystalline egg albumin was inoculated into a portal vein radicle of rabbits which had been previously rendered Arthus-positive by repeated subcutaneous injections of the same protein. Most of the animals survived this procedure and were sacrificed from 2 hours to several months afterward.

The livers of these animals present striking changes. Macroscopically the 24 hour lesions are yellowish gray, round, well demarcated areas, measuring from pin-point to 1 mm. in diameter. They are present throughout the parenchyma but are more numerous immediately beneath the capsule and bear no apparent relationship to lobular architecture. Microscopically the lesions are either midzonal or peripheral. They may be very small, affecting only about 20 to 25 cells, or they may involve almost the entire lobule. More than one lesion may be present in the same lobule. The affected parenchymal cells show coagulative necrosis as early as 8 hours, and there is an early infiltration of polymorphonuclear leukocytes into the involved area. Within 24 hours the hepatic cells in the affected areas have lost their integrity, the cords having become homogeneous granular masses containing an occasional faded nucleus. An important characteristic of the lesion is the constant absence of hemorrhage because the connective tissue framework of the lobule remains intact. An occasional thrombosed portal vein is present, but this finding is so rare in comparison to the large number of lesions that it is probably secondary to the massive necrosis of the hepatic cells. Further degenerative and reparative processes are present in the older lesions.

These studies show that the mechanism of allergic inflammation in the liver is similar in all essential respects to that which occurs in the skin. It would seem that the response is due primarily to the union of antigen with its specific antibody on or within the liver cells.

ACUTE ALLERGIC INFLAMMATION OF THE LUNGS. Paul R. Cannon and (by invitation) Theodore E. Walsh and Charles E. Marshall, Chicago, Ill.

Abstract. The Arthus phenomenon has aided greatly in the understanding of certain aspects of allergic inflammation. Its demonstration in the skin, however, has tended to overemphasize the vascular effects with the resulting gangrenous necrosis. This apparent overemphasis is probably due to the fact that in the skin antigen and antibody combine in a comparatively small volume of compact collagenic tissue. We therefore decided to study this phenomenon in the lungs where the antigen could be dispersed over a larger surface and where the ensuing vascular reactions could be readily observed.

Rabbits made hypersensitive to crystalline egg albumin, and normal controls, were given simultaneous intranasal instillations of a solution of crystalline egg albumin. At varying intervals the animals were sacrificed by air embolism and their lungs were fixed *in situ* in Zenker-formalin and sections stained with hematoxylin and eosin. In some instances animals passively sensitized by injection of specific antiserum were similarly treated.

The results are as follows: In the hypersensitive animals acute edema, alveolitis, bronchitis and pneumonic consolidation occurred regularly; the severity of these conditions depended upon the degree of sensitivity of the animal and the amount of material entering the lungs. There was a marked increase in lymph flow in the perivascular lymphatics with acute hemorrhage at times. Acute arteritis and phlebitis occurred in some instances, and mural thrombosis was occasionally seen, particularly in small arteries and in pulmonary veins. Infarction developed in some areas of consolidation. In contrast with these findings the effects in the control animals were insignificant.

These experiments indicate that the primary effect of the antigen-antibody reaction in the lungs was an increased capillary permeability accompanied by acute edema, hemorrhage and leukocytic accumulation. When the vascular injury was more severe arteritis or phlebitis, with occasional mural thrombosis, occurred. The fact that the changes produced in passively sensitized animals differed only in degree from those in animals actively sensitized suggests that the tissue reactions were due to a changed property of the serum rather than of the tissues themselves. The conclusion is reached, therefore, that these tissue reactions have resulted from the combination of antigen and antibody within cells, particularly those of the blood vessel walls. The severity of the reaction depends, presumably, upon the varying intensities of the antigen-antibody union.

Discussion of papers by Drs. Hartley and Lushbaugh, and Cannon, Walsh and Marshall

(Dr. Benjamin J. Clawson, Minneapolis, Minn.) I should like to raise a question about the term "allergic inflammation." I have used it myself and I think it has come to be thought of as some specific anatomical type of inflammation, just as one would speak of tuberculous inflammation. While this has been shown to be due to a hypersensitive reaction, I should like to ask Dr. Cannon if he would put himself on record as diagnosing "allergic inflammation," if he saw it on a slide and knew nothing about the history of the case. I should also like to have his opinion about this arteritis — whether it has any similarity to the type of arteritis which is described by Pappenheimer and Von Glahn as specific for rheumatic fever.

(Dr. Ernest M. Hall, Los Angeles, Cal.) I should like to ask Dr. Hartley and Dr. Cannon whether they found any changes in the arteries of the heart similar to the changes in the liver and the lungs, with fibrinoid necrosis of the vessel wall and swelling about the vessels, and so on. We have seen these changes after the intravenous injection of horse serum. I should like to ask Dr. Hartley whether the scars resulting from the necrosis of the liver are permanent. I would expect they would not be.

(Dr. Virgil H. Moon, Philadelphia, Pa.) I am moved to comment on these contributions because they merge over into the field, or are part of the field, which has afforded me much stimulating pastime in recent years. The formation of edema incident to the introduction of the antigen in these cases illustrates a method for the production of edema experimentally, and I believe Dr. Cannon is correct in his interpretation of it as resulting from damage to the vascular endothelium. Dr. Opie has been quoted several times to the effect that cells are damaged when antigen and antibody meet within them. Endothelial structures are cells. They likewise are damaged by this type of meeting. When damaged, they allow the plasma to escape into the

interstitial spaces. Frequently, as in some of the sections shown by Dr. Cannon, there is not only edema but also extravasation of red cells incident to capillary damage. These features, shown in experiments in which the antigen was introduced by insufflation, are essentially identical with those we have produced by introducing the antigen intravenously. In the latter case the edema was diffuse throughout the lung tissues, whereas when it was introduced in the bronchial passages the resulting edema and inflammation were most marked around these structures. I have been greatly interested in both these presentations.

(Dr. Joseph Tannenberg, Albany, N. Y.) I should like to ask Dr. Cannon if he also made experiments in which the same animals were treated with various kinds of proteins. Some years ago I sensitized rabbits repeatedly to various bacterial proteins or to horse serum. Upon intravenous injection of the specific antigen I observed the same lesions Dr. Cannon has demonstrated, especially the arteritis and the edema around the pulmonary arteries and edema of the arterial walls. But I also obtained the same lesions when normal rabbits were repeatedly intravenously injected with distilled water. These injections were made more than three or four times with intervals of several days.

(Dr. Malcolm H. Soule, Ann Arbor, Mich.) Dr. Cannon, you are familiar with the fact that histaminase influences the anaphylactic reaction in the guinea pig. Do simultaneous injections of this substance with the shocking antigen have any effect in these experiments?

(Dr. Howard T. Karsner, Cleveland, Ohio.) I recognize the difficulty of identifying eosinophils in material of this sort. I think, however, inasmuch as these cells have been thought of as being important in reactions of this kind, that perhaps Dr. Cannon might let us know if they have participated to any great extent in the exudation, particularly that in the blood vessel walls.

(Dr. Hartley.) In answer to Dr. Hall's question about coronary artery changes, we studied the hearts, lungs, kidneys and spleens of all of the animals, but did not see any changes in any organ except the liver. I suspect that the antibodies which are present on or within the hepatic cells localize the antigen in the liver before it can reach the general circulation. With regard to the question about fibrosis, the lesions that you saw were about 1 month old. From the appearance of the fibrotic areas I feel quite sure that such a lesion would have persisted indefinitely. However, I might emphasize again that such a fibrotic lesion is only occasionally seen, the usual end result being complete disappearance of the lesion.

(Dr. Cannon, closing.) In answer to Dr. Clawson's question concerning the use of the term "allergy," it is impossible, of course, to get much agreement as to the connotations of the term. I use it essentially as suggested by von Pirquet, *i.e.* altered reactivity to a presumably known sensitization. I believe that there has been too much emphasis upon classification in this field and that the term allergy should be used in a broad sense.

In regard to Dr. Clawson's second question, the diagnosis of allergic inflammation histologically, I do not have much confidence in my own ability to diagnose it. I regard allergic inflammation mainly as an intensification of the normal inflammation, with the additional elements of necrosis, eosinophilic accumulations and more energetic repair.

As to the possible relationship of allergic inflammation as shown here to rheumatic arteritis and periarteritis nodosa, Libman-Sacks' disease, and so on, one should remember that we are using only one type of purified protein. It is conceivable that many other antigenic materials may cause similar reactions, but differing because of variations in their chemical constitution and molecular size.

In reply to Dr. Tannenberg, I would say all of these experiments were acute ones. We were simply interested in studying the vascular changes in the lungs with particular reference to the problem of pulmonary edema and the mechanism of acute allergic inflammation.

We have not tried histaminase in any of these studies.

It is difficult to be sure about eosinophils in the rabbit. The pseudo-eosinophils were conspicuous, particularly in the acute inflammation, but it was difficult to determine the extent of accumulation of true eosinophils. We have thought that there was an increased accumulation of these eosinophils with their large, ovoid, eosinophilic granules, but we have not emphasized that point very much because of the difficulties inherent in their identification in the rabbit.

MECHANISM OF LEUKOCYTOSIS WITH INFLAMMATION: THE NATURE OF THE LEUKOCYTOSIS-PROMOTING FACTOR. Valy Menkin, Boston, Mass.

Abstract. Earlier studies by the writer (*Am. J. Path.*, 1940, **16**, 13) have demonstrated in exudates the presence of a leukocytosis-promoting factor which offers an adequate explanation for the mechanism of leukocytosis accompanying inflammatory processes. This factor bears evidently no relation to leukotaxine, the nitrogenous substance capable *per se* of explaining increased capillary permeability and leukocytic migration in areas of acute inflammation. The leukocytosis-promoting factor has been shown to be present in exudates of dogs. More recently its presence has likewise been identified in the exudative material of rabbits. The material is thermolabile and is essentially non-diffusible. The present studies indicate that it seems to be either a globulin or at least that it seems to be associated with the pseudoglobulin fraction of exudates. The purified material yields positive biuret, ninhydrin, and Adamkiewicz tests, the last being for the presence of the indol nucleus. This would support the view concerning the protein nature of the factor. The persistence, however, of a positive Molisch test does not preclude the possibility of a carbohydrate as a prosthetic group. Phosphorus is also present in the purified material.

The methods utilized for the extraction of the leukocytosis-promoting factor can be stated somewhat as follows: The whole or cell-free exudate is dialyzed against tap water. The precipitated euglobulin fraction is found to be essentially inactive and it is consequently discarded. The albumin fraction is separated from the pseudoglobulins by subsequent precipitation of the latter at one-half saturation of ammonium sulphate. The albumins are found completely ineffective in altering the number of circulating leukocytes, the average rise being 2.7 per cent. The untreated exudative material induces in dogs an increase in white blood cell counts averaging 77.2 per cent. The maximum increase in the normal leukocytic variation of dogs is found to average 26.2 per cent. The euglobulin fraction obtained either by dialysis or by precipitation of the exudate at one-third saturation of ammonium

sulphate yields an average leukocytic rise of 29.1 per cent and 21.3 per cent respectively. On the other hand, the globulin fraction obtained by precipitation at one-half saturation of ammonium sulphate induces an average increase of 88.9 per cent in the number of circulating leukocytes. This indicates that the leukocytosis-promoting factor (LPF) seems to be associated with the pseudoglobulin fraction of exudates. The purified substance is conveniently obtained in a desiccated state as follows: The exudate is treated with ammonium sulphate at one-half saturation. The dialyzed precipitated mixture is vigorously shaken with ether, presumably to remove the lipoprotein fraction. The residual material is reprecipitated with ammonium sulphate at one-half saturation, dialyzed, and evaporated to dryness *in vacuo* at about 35 C. Blood serum fractionated in a manner similar to exudates fails to yield any active fractions. Finally, the leukocytosis-promoting factor seems far more potent than a pentnucleotide preparation (obtained from Smith, Kline and French Laboratories). This, therefore, suggests additional clinical possibilities for a substance liberated at the site of injury and which *per se* offers a reasonable explanation for the leukocytosis frequently associated with inflammation.

COMPARISON BETWEEN POLYMORPHONUCLEAR LEUKOCYTES AND MONOCYTES IN REGARD TO THEIR CHEMOTACTIC BEHAVIOR. Dale Rex Coman, Philadelphia, Pa.

Abstract. The chemotactic responses of polymorphonuclear leukocytes and monocytes to *Staphylococcus albus*, tubercle bacillus (Bovine III) and to an aluminum silicate (Lloyd's reagent) were studied in tissue cultures. Preparations for studying the polymorphonuclear leukocytes consisted of explants of buffy coats from the blood of white rats, in autoplasma. The monocyte preparations consisted of explants of rat omentum in autoplasma. The substance to be tested for its chemotactic effect was placed in a small clump (1 mm. in diameter) upon a coverslip. The explants were then placed about 0.5 mm. from this clump and the plasma was added in as thin a film as possible. The preparations were sealed to prevent evaporation and incubated at 37 C. for 18 to 24 hours. After this time the distribution of the cells in relation to the test material was recorded by microphotographs. If the majority of cells had moved toward the test material it was taken to indicate positive chemotaxis. If the cells had moved away from the test material it was taken as an indication of negative chemotaxis. When the cells showed an even distribution around the explant it was considered that no chemotactic response had occurred.

It was thus demonstrated that polymorphonuclear leukocytes were attracted by, *i.e.* showed positive chemotaxis to, both *Staphylococcus albus* and the tubercle bacillus, and that they were repelled by, or showed negative chemotaxis to, the aluminum silicate. In contrast to these responses of the polymorphonuclear leukocyte, the monocytes, under similar conditions, failed to show chemotaxis in the presence of each of the three test materials. The movement of the monocytes continued to be at random, uninfluenced by the presence of either staphylococci, tubercle bacilli or aluminum silicate. Phagocytosis of tubercle bacilli and staphylococci both by polymorphonuclear leukocytes and by monocytes was observed whenever there was contact between cell and organism.

It is concluded from these experiments that the monocyte differs strikingly from the polymorphonuclear leukocyte in its chemotactic responses. The direction of movement of the monocyte is not affected by either of two organisms or by a chemical substance, each of which excites a strong chemotactic response in the polymorphonuclear leukocyte. The presence of the monocyte in large numbers in certain inflammatory foci *in vivo* may depend upon mechanisms other than chemotaxis. For instance, these cells may enter such areas through random motion and then become trapped. On the other hand, the monocyte may be chemotactically attracted by substances present in the inflammatory focus, which have not yet been tested under our experimental conditions.

Discussion

(Dr. C. V. Weller, Ann Arbor, Mich.) I should like to ask Dr. Coman if he has any explanation for the solitary exception in the group of 30 experiments relating to staphylococci and polymorphonuclear response. Why, in that large group, should there be a solitary example of contrary behavior?

(Dr. Coman, closing.) These fields are recorded, whether the conditions for recording are ideally favorable or not. In other words, some of these explants are presumably too far away from the target to be affected strongly by it, and perhaps during the incubation period there was insufficient of the chemotactic substance thus far out in the plasma, and the cells are therefore actually indifferent. However, all cases are included, and even a slight movement one way or the other was regarded as positive or negative; in other words, the examples shown were very marked responses, whether strongly positive or negative. In some the cuff of cells would grow out in a more or less indifferent manner, but the attempt was made to measure the microphotographs with calipers to decide whether or not for mathematical purposes we could consider it as a positive or a negative response.

A Cutaneous and Precipitation Antiserum Reaction in Experimental (Virus) Poliomyelitis of Monkeys. Edward C. Rosenow, Rochester, Minn.

Abstract. Three-hundredths of a cc. of a 10 per cent solution of the water-insoluble (euglobulin) fraction of the serum of horses hyperimmunized with streptococci from poliomyelitis, and as controls, the same material but from serums prepared with streptococci from diseases other than poliomyelitis, and normal horse serum are injected intracutaneously. The serum of monkeys is superimposed in precipitin tubes on the clear whole antistreptococcal serums. In the cutaneous test an edematous and sometimes erythematous reaction occurs in 10 to 30 minutes, surrounding the point of injection of the poliomyelitis antiserum and with little or no reaction to control injections. The precipitation reaction consists of clouding at the interphase and occurs regularly with the poliomyelitis antiserum during the acute stage of poliomyelitis, disappearing with recovery. Reactions of the skin and serum of healthy monkeys are uniformly negative.

The pseudoglobulin (antitoxic-antibody) fraction is not suitable for the cutaneous test; when it is injected intracutaneously in mixture with the euglobulin fraction (2:1) it interferes with the cutaneous reaction; and when injected intramuscularly in therapeutic amounts (0.5 cc. per kilo), it causes

reactivity of the skin to reinjection of the poliomyelitis euglobulin, and the precipitation reaction to disappear promptly. Concomitantly it has beneficial effects in reducing the severity of symptoms and mortality. Similar therapeutic injections of the pseudoglobulin fraction of the arthritis antistreptococcus serum have no effect on the cutaneous and precipitation reaction to the poliomyelitis euglobulin and the course of the disease. Identical cutaneous and precipitation reactions to the poliomyelitis euglobulin and the whole antiserum, respectively, are obtained in monkeys following intracerebral inoculation of dead poliomyelitic streptococci. This is not the case following intracerebral inoculation of monkeys with comparable numbers of dead streptococci from cases of arthritis.

SOME PROPERTIES OF THE ENCEPHALOPATHIC AGENT IN PRIMATE BONE MARROW (THE GORDON AGENT). Lester S. King, Princeton, N. J.

Abstract. The nature of the encephalopathic agent present in the bone marrow of monkeys has been studied. It is readily soluble at a pH of 7.4, as well as at the low pH of 2 and 3. The point of minimal solubility is pH 4.2. Heat inactivation is produced by a temperature of 75 C. acting for 15 minutes. The agent is digested by pepsin. It exhibits a slight degree of sedimentation with the air-driven centrifuge. It is non-dialyzable. It is precipitated from solution (pH 7.4) by ammonium sulphate over a range of 0.4 to 0.8 saturation. These findings all suggest that the agent is a protein.

Discussion

(Dr. Edward C. Rosenow, Rochester, Minn.) The experiments just reported remind me of the results of experiments which I performed years ago in connection with various autolysates of pneumococci and streptococci, in which among other things it was found that animals often died after a period of incubation of from 7 to 14 days following intraperitoneal or other injections. This suggested that death in some way may have been due to liberation of "anaphylotoxin" from interaction of antibody and undigested antigen or protein. Could it be that the protein from bone marrow, considered as the pathogenic agent, resists digestion until the animal becomes sensitized, and that the symptoms and lesions are attributable to an allergic reaction between antibody and previously undigested protein or antigen? Have other tissues yielded proteins similar in their action?

(Dr. Paul E. Steiner, Chicago, Ill.) I should like to ask Dr. King if he has any direct evidence that this agent from normal primate bone marrow is identical with the "Gordon agent." As I understand it, the evidence in the literature at present is indirect evidence. Is there any direct evidence?

(Dr. King, closing.) In answer to Dr. Rosenow's question, in the literature and in my own experience many tissues from many different animal species have been studied. The consensus of opinion seems to be that this agent can be obtained principally from primate eosinophils. These are not the exclusive agent since a similar phenomenon can be obtained with mouse and chicken spleens, which are quite different from primate material, as well as from pure pus suspensions. So many organs have been tested from so many different species that there seems to be a definite specific relation of this particular reaction to certain blood cells, especially, though not exclusively, the eosinophils.

In relation to the identification of the bone marrow of the monkey with the original Gordon agent, I think the weight of evidence shows that in the original Gordon test this reaction is due to the eosinophils. Whether in the bone marrow from the monkey the eosinophils are specifically responsible has not been directly investigated. However, I have tested extracts of lymph nodes from cases of Hodgkin's disease and have compared them with the bone marrow extracts, not only in the rabbit, which Gordon used, but also in the guinea pig. The clinical reaction, the incubation period and the pathological reaction have been identical. This is indirect evidence of the identity of the Gordon factor and the monkey factors, but until we have the agents in pure crystalline form we cannot be absolutely certain.

PANCREATITIS IN ACUTE AND CHRONIC ALCOHOLISM. Eugene Clark, New York City.

Abstract. A report of 44 cases of pancreatitis occurring in acute and chronic alcoholism, gathered during a 5 year period from autopsies at the Bellevue Hospital and the office of the Chief Medical Examiner, New York City, is presented. Pathological alterations of the gall bladder or bile ducts were absent. An impression of the frequency of such lesions may be gained from the occurrence of 27 of these cases among approximately 150 cases of acute and chronic alcoholism autopsied at Bellevue Hospital. In 23 of the 44 cases the pancreatic inflammation, necrosis and hemorrhage were of such magnitude as to be considered the probable cause, or an important contributory cause of death.

In 36 cases microscopic studies were carried out. The lesions were classified as acute in 19, acute and chronic in 5, chronic in 9, and fat necrosis was present in 3, these categories suggesting only the predominant lesion, for in many of the acute cases some healed foci were found, and fat necrosis was present in all but 7 cases. In 24 the liver was large and fatty, and in 17 cirrhosis was present.

Metaplasia of the duct epithelium was present in 4 cases.

The lesions were seen only in individuals who had experienced a prolonged alcoholic debauch; in all the cases of massive acute pancreatitis the onset was preceded by a period of acute alcoholic intoxication.

INTRAHEPATIC FAT NECROSIS CAUSED BY PANCREATIC REFLUX. Walter Schiller, Chicago, Ill.

Abstract. Pancreatic and hepatic biliary ducts may be transformed into one communicating system by obstruction of the outlet, *i.e.* the papilla of Vater. The most frequent cause of obstruction is stones of the common duct; however, spasm of the sphincter of Oddi and edema of the duodenal mucosa must be taken into consideration. Reflux of bile into the duct of Wirsung may cause acute hemorrhagic pancreatitis. Reflux of the pancreatic secretion into the bile ducts has been described as the etiological factor for certain liver changes of different but unspecific character. That reflux has been proved by amylase determination of the bile. The presence of amylase is indicative of high titer only inasmuch as bile normally contains amylase in low titer; whereas the presence of lipase in the bile is conclusive evidence for pancreatic reflux, since normal bile contains no lipase. In a case of acute hemorrhagic pancreatic necrosis combined with an old hypertrophic periportal

cirrhosis of the liver, numerous foci of typical fat necrosis were found, not only in the pancreatic and the peripancreatic fat tissue, but also scattered all over the parenchyma of the liver. The incidence of these intrahepatic foci of fat necrosis was higher around the porta hepatis than in the peripheral parts of the liver.

Microscopically the foci show fatty acid crystals which give a positive Benda reaction. The pancreatic duct contains several small soft stones; one of them evidently had blocked the papilla of Vater and thus transformed the pancreatic and hepatic duct into one communicating system. Through the bile ducts the pancreatic lipase had reached the fatty degenerated liver cells.

THE SUBCUTANEOUS NODULES OF RHEUMATOID ARTHRITIS AND RHEUMATIC FEVER. A PATHOLOGICAL STUDY. Granville A. Bennett and (by invitation) J. Wallace Zeller and Walter Bauer, Boston, Mass.

Abstract. A comparative study of subcutaneous nodules of varying ages removed from patients with rheumatoid arthritis and from patients with rheumatic fever was undertaken in order to describe the characteristic features of these lesions and to determine, if possible, whether they are disease specific. A few nodules from the sclera (scleromalacia perforans), pericardium, pleura and synovial tissues of two patients with rheumatoid arthritis were also studied. The changes in these lesions were similar for the most part to the nodules that were situated subcutaneously.

Although it was possible in most instances to distinguish the rheumatoid arthritis nodule from that of rheumatic fever, it was apparent that similar or identical structures and cytological features were observed in both. Differentiation was generally possible because one or more of the pathological alterations predominated. The changes observed in the rheumatic fever nodule were interpreted as being due chiefly to injury of small blood vessels with subsequent exudation of plasma and blood cellular constituents into the connective tissues. In addition, small focal lesions similar or identical with the myocardial Aschoff nodule were frequently seen. These features differed from those of the rheumatoid arthritis nodule in which marked proliferation and degeneration of the connective tissue predominated, and in which Aschoff-like nodules were not observed. Exudation was rarely a prominent feature of these lesions. Such findings afford an explanation for the differences in time of appearance, duration and size of the nodules in these diseases. The rheumatic fever nodule, being chiefly an exudative phenomenon, might be expected to appear more quickly and resolve more promptly, leaving little or no residuum. The continued proliferation and necrosis of the rheumatoid arthritis nodule, on the other hand, would explain its longer duration and larger average size. The process of repair would require more complicated mechanisms, cicatrization and calcification occurring much oftener.

The observed morphological differences between the nodules of rheumatoid arthritis and rheumatic fever suggest that they are not necessarily the result of the same injurious agent. The clinical and pathological differences in rheumatoid arthritis and rheumatic fever also favor such an interpretation.

Further studies of these readily accessible subcutaneous nodules by new and untried methods should be carried out in the hope that more may be learned concerning their pathogenesis and cause. The present comparisons have yielded information that should be useful in differential diagnosis.

Discussion

(Dr. Irving Graef, New York City.) I should like to ask Dr. Bennett if he has made fibrin stains on paraffin sections of these nodules, and what they showed if they were made.

(Dr. Bennett.) A number of staining methods were employed. These included, in some instances, stains for the demonstration of fibrin. In the altered connective tissues of the rheumatic fever nodules material was frequently observed which stained in a manner we believed to be typical of fibrin. In reticulum stains and certain connective tissue stains the swollen connective tissue strands also contained fibrillar material which we interpreted as collagen fibrils.

(Dr. Graef.) I wonder whether Dr. Bennett would care to comment on the use of the term "fibrinoid degeneration" in this connection. It is a term which has plagued me and I know has plagued many other people. In two different studies in which we thought we were dealing with fibrinoid degeneration — one in the walls of arteries and the aorta, associated with thrombi, and the other in rheumatic hearts containing auricular thrombi — such so-called fibrinoid degeneration was actually fibrinous infiltration of subendocardial or subendothelial tissue; further, that the deposits of fibrin separated preexisting collagenous bundles and were independent of true collagenous swelling. I believe too much attention has been paid to the occurrence of such material among collagenous bundles, for it stains like fibrin when so treated, while altered collagen does not. Its occurrence has even been interpreted as part of the so-called rheumatic complex, or the primary lesion of rheumatic inflammation. I wonder, therefore, if Dr. Bennett would care to comment on the precise use of the term in relation to descriptions of rheumatic fever. In the *Annals of Rheumatic Diseases*, Volume 1, No. 3, July, 1939, published in Great Britain, there was an article by Aschoff describing the submiliary myocardial nodule, in which he pointed out that it was in no way essential that the formation of the nodules should be preceded by fibrinoid degeneration of the ground substance.

(Dr. Bennett, closing.) The term "fibrinoid degeneration" as used in this report is not a term of my selection. I agree with Dr. Graef that it has received more attention than it probably deserves. I believe that this type of reaction frequently represents a non-specific type of inflammatory change in connective tissue in which the collagen fibers are separated by exudate, and I would prefer to designate the type of change I showed in some of the rheumatic fever nodules as "plasma soaking" rather than as fibrinoid degeneration.

ULCERATING VALVULAR LESIONS IN SUBACUTE BACTERIAL ENDOCARDITIS DUE TO STREPTOCOCCUS VIRIDANS. REPORT OF A CASE. Alfred F. Knoll (by invitation), Pittsburgh, Pa.

Abstract. Aside from the writings of Libman, ulcerating valvular lesions in subacute bacterial endocarditis have received scant recognition in the literature. When ulceration does occur it is usually in the aortic flap.

A case is reported in which a bicuspid aortic valve presented a large vegetation associated with an aneurysmal outpouching and perforation of the posterior cusp. Blood cultures showed pure growths of *Streptococcus viridans*. Microscopically the vegetations showed recent healing and healed stages of

inflammation. A focal embolic glomerulonephritis was present. The difficulty of classifying the combination of lesions under the term subacute bacterial endocarditis is discussed.

ETIOLOGY OF THE CALCIFIED NODULAR AORTIC VALVE. Ernest M. Hall, and (by invitation) Tsutayo Ichioka, Los Angeles, Cal.

Abstract. Thirty-one unselected cases of calcific aortic stenosis were studied largely from the anatomical standpoint. The object was to discover evidences of rheumatic infection, or criteria favoring Mönckeberg's aortic sclerosis. Forty-eight per cent of the whole group had a history of rheumatic infection in childhood or in early adult life. There were 12 cases in which the mitral as well as the aortic valve was involved. The remaining group of 19 cases had only the aortic valve affected. Six standard blocks, according to the method of Gross, Antopol and Sacks, were taken from each heart for microscopic study.

In the first group of 12 patients the ages ranged from 38 to 86 years. Males predominated 3 to 1. Seven out of 12 had a history of rheumatic infection (53.3 per cent) and Aschoff bodies were found in an equal percentage of cases. Seventy-five per cent showed chronic inflammation of the aortic valve ring. All but 2 had some evidence of healed pericarditis, and all exhibited various other stigmas of rheumatic infection. Arteriosclerosis of the aorta was fairly well marked in 2 cases and moderate in 3.

In the group of 19 cases with a single aortic lesion the ages ranged from 20 to 81 years. Males predominated nearly 4 to 1. A history of rheumatic infection was obtained in 8 cases, or 42 per cent. Aschoff nodules were seen in the myocardium in 9 patients (47.4 per cent). Chronic inflammation of the aortic ring was seen in 16 cases, or 84.2 per cent.

Evidence of healed pericarditis was present in 6 cases. All cases revealed other stigmas of rheumatic infection. By these we mean arteritis, "fibrinoid" swelling and necrosis of the small blood vessels, mononuclear cell infiltration of the myocardium, edema, perivascular scarring, endocardial thickening, and so on. Eight of this group presented bicuspid aortic valves without other evidence of congenital changes. This condition is regarded as strongly suggestive of rheumatic involvement. Three patients had marked arteriosclerosis of the aorta, and 3 had only moderate changes.

Two or more important evidences of rheumatic involvement (positive history, Aschoff nodules, chronic inflammation of aortic ring, bicuspid valve) were found in each of the 31 cases, in addition to variable numbers of the lesser stigmas. These facts, together with an absence of any substantial evidence favoring Mönckeberg's aortic sclerosis, cause us to conclude that all of our cases appear to be of rheumatic genesis.

INFARCTION OF THE AURICLES OF THE HEART. Edward H. Cushing (by invitation), William B. Wartman, and Eugene Stanton (by invitation), Cleveland, Ohio.

Abstract. A pathological and experimental study was made of infarction of the auricles of the heart. The incidence of the lesion was about 0.01 per cent of 2350 autopsies. The race, sex and age incidence of the infarcts resembled those found in ventricular infarction. Over 80 per cent were located in the right auricle, most of them being in the appendage. The gross and micro-

scopic appearance of auricular infarction was similar to ventricular infarction. In 81.2 per cent of the cases there was accompanying mural thrombosis of the auricular endocardium.

The functional effects of auricular infarction were studied in dogs in which the auricular arteries had been ligated. Although the blood supply of the dog's heart is not comparable with that of the human, ligation of the auricular arteries resulted in infarcts which resembled those seen in human autopsy material. Several of the dogs survived the operation for long periods of time and at autopsy showed remote infarcts.

It is suggested that the prevalence of infarction in the right auricle and especially in the auricular appendage may be due to the presence of venous blood in the right side of the heart.

Discussion

(Dr. W. C. Hueper, Scarsdale, N. Y.) I wonder whether Dr. Wartman has any information on whether any of his cases were treated with digitalis preparations before death. We have conducted quite recently some studies on experimental chronic digitalis poisoning in animals (cats and guinea pigs) and I have seen in several of these animals hemorrhage and hemorrhagic infarction in the right auricular wall which is identical with the pictures shown here.

(Dr. Kornel L. Terplan, Buffalo, N. Y.) I should like to ask whether any special attention was given to the blood supply of the right side of the heart in cases where infarction of the right auricles was found. Anomalies in the anlage of the right coronary artery, especially small accessories, are relatively frequent.

(Dr. Wartman, closing.) In answer to Dr. Hueper, I have no information as to whether or not these patients were treated with digitalis. Dr. Hallorhan and Dr. Feil of Cleveland, who have been interested in this question, are going over the material in regard to the effect of digitalis, but at present I cannot answer the question.

In reply to Dr. Terplan's question, many of these cases were discovered accidentally, so that in many of them the auricular arteries were not examined. Recenty we have examined the auricular arteries, but in no case have we found an occluded artery on gross examination. However, microscopically it is possible in some cases to demonstrate thrombosis of the small branches of the arteries. I would agree with Dr. Terplan that anomalies of the auricular arteries are common.

CARDIAC LESIONS IN CHRONIC INFECTIOUS (RHEUMATOID) ARTHRITIS. A. H. Baggenstoss and (by invitation) E. F. Rosenberg, Rochester, Minn.

Abstract. In a study of 25 cases of chronic infectious arthritis at autopsy cardiac lesions were found in 20. Lesions resembling those produced by rheumatic fever were found in 14 (56 per cent) and non-rheumatic lesions or lesions of doubtful nature were present in 6 (24 per cent). Normal hearts were present in only 5 instances (20 per cent).

Non-rheumatic lesions were as follows: coronary sclerosis with infarction of the myocardium in 2 cases; and cardiac hypertrophy, non-specific fibrinous pericarditis, hydropericardium and fibrous obliteration of the pericardial sac in 1 case each, respectively.

The rheumatic lesions were grossly demonstrable in all but 1 of the 14 cases. Gross lesions were present in the mitral valve in 10 cases. Severe stenosis of the mitral valve was present in 2 cases and moderate stenosis occurred in 2 others. The aortic valve revealed gross evidence of rheumatic injury in 5 cases, and there was rheumatic injury of the tricuspid valve in 2. The pericardium was involved in 5 cases. The exudate was fibrinous in 3, fibrinopurulent in 1, and in 1 instance the pericardial sac was obliterated by fibrous adhesions.

By histological classification the predominant lesions were subacute in 3 cases, chronic in 7 and inactive in 4. Typical myocardial Aschoff bodies were present in all cases in which there were subacute lesions; in 2 of them small abscesses were present in the myocardium also. In the group of 7 cases in which chronic lesions were found, evidence of myocardial injury was present in all. Aschoff bodies in the pericardium, valves or myocardium were present in all but 2 of the cases of this group. A significant feature of this group was the fact that in 5 cases evidence of recent acute rheumatic damage was found in addition to the chronic inflammation. The acute lesions were in some instances superimposed on inactive or chronic inflammatory processes, and in other instances they appeared in a previously uninvolved portion of the heart. In the group of 4 cases in which inactive lesions were found, the main histological evidence of previous inflammation was fibrosis and vascularization of the leaflets of the valve.

Only 7 of the 14 cases in which rheumatic cardiac lesions were found presented signs or symptoms which were indicative of heart disease existing during the life of the patient. The facts, however, that cardiac hypertrophy in which the heart weighed more than 300 gm. occurred in 11 of the 14 cases in which rheumatic cardiac lesions were present, that the cardiac injury played an important rôle in the cause of death of the patient in 7 of the cases, and finally that there was histological evidence of activity in the rheumatic lesions at the time of death of the patient in all but 4 of the 14 cases, are indicative of the importance of these lesions.

(Dr. Baggenstoss.) The material included all cases of rheumatoid arthritis there were cardiac lesions grossly and histologically indistinguishable from those produced by rheumatic fever suggests a definite relationship between these two diseases.

Discussion

(Dr. B. J. Clawson, Minneapolis, Minn.) This group of cases reported by Dr. Baggenstoss presents several interesting things. I want to mention two: (1) the large number of cases of chronic infectious arthritis coming to autopsy; and (2) the high incidence of the association of the stigmas of rheumatic infection. I think we can have no doubt about these being valvular deformities and Aschoff nodules. I should like to ask Dr. Baggenstoss to explain how he selected his material. Was this selected material? Were the cases diagnosed rheumatoid arthritis or heart disease?

(Dr. Baggenstoss.) The material included all cases of rheumatoid arthritis in which an autopsy had been performed. In each instance the diagnosis was a clinical diagnosis, and in each instance it was made by a member of the Section on Arthritis at The Mayo Clinic. All patients included in this study had chronic polyarticular inflammation, generally insidious in onset, although occasionally acute. In each instance the arthritis had run a progressive

course over a period of months or years. There was always some degree of articular crippling. Periarticular and synovial thickening were common, and frequently effusions were present. Some degree of fibrous ankylosis was common. Roentgenograms in the well developed cases revealed swelling of periarticular soft parts, narrowing of the joint space, atrophy of epiphyseal bone, destruction of subchondral bone and later marginal lipping. Systemic manifestations such as slight fever, loss of weight and hypochromic anemia were usually present. If there was any doubt as to whether the case was one of rheumatoid arthritis or rheumatic fever, it was not used.

(Dr. Clawson.) Do you have the ages of the patients?

(Dr. Baggenstoss, closing.) The average age at the onset of the disease was 34 years, and the average age at death was 42 years.

EXPERIMENTAL HYPOTENSIVE ARTERIOSCLEROSIS IN RATS. Wilhelm C. Hueper, New York City.

Abstract. On the premise that the fundamental and general direct causative mechanism of arteriosclerosis is represented by an impairment of the nutrition of the vascular wall, rats 2 to 3 months old were treated for 5 to 7 months with a hypotension- and thus hypoxemia-producing agent — erythrol tetranitrate. The histological examination of the internal organs of these animals showed sclerotic lesions of the arteries of the heart, lung, kidney and testes, while the cerebral vessels exhibited endothelial proliferation, perivascular edema and fibroblastic proliferation. There were focal glioses, small necroses, edema and vacuolar ganglion cell degeneration in the brain. The meninges were edematous, congested and cellular.

DISSECTING ANEURYSM OF THE AORTA. J. S. McCartney, Minneapolis, Minn.

Abstract. A survey of the approximately 35,000 postmortem examinations in the department of pathology of the University of Minnesota revealed 107 cases of dissecting aneurysm of the aorta. In this group of aneurysms 14 gross specimens were found where there was gross evidence of chronicity and healing. The ages of these individuals ranged from 30 to 76 years; 10 were males and 4 females. In 7 the aneurysm was the cause of death through rupture and hemorrhage. In 8 there was clinical evidence of hypertension and in 10 cardiac hypertrophy.

The linings of these dissections were for the most part smooth and shiny with yellow foci of atheromatous change on this shiny surface. In a number there was also thrombosis to a greater or lesser extent.

Microscopic examination of the split surfaces showed regeneration of the intima with a large amount of connective tissue. In some there was evidence that the regeneration was due to organization of an overlying thrombus; in others it appeared to be a simple proliferation of connective tissue covering a denuded surface. The recovered surface was usually much thicker than the normal intima. The surfaces immediately in contact with the blood usually showed a distinct layer of endothelial cells. In the newly formed intima fatty changes were commonly present and in a number of instances the atheromatous change was more marked on the new surface than on the intima of the original lumen. The finding of the atheromatous changes in the newly formed intima suggests a number of factors which may be responsible

for this change, such as pressure, environment and nutrition, and not merely the question of the age of the intima itself.

In only 5 of the cases was there any clinical evidence as to the duration of the dissection. The longest duration was 4 years and 10 months, the shortest 75 days. The last case showed three healed aneurysmal sacs with connecting dissection. In 2 cases there was evidence of syphilis.

Discussion

(Dr. C. V. Weller, Ann Arbor, Mich.) I should like to ask Dr. McCartney if he was impressed in connection with the protocols of these cases with any preponderance of a physical type. I have had several experiences that have led me to think that dissecting aneurysm is especially likely to occur in rather asthenic, dolicomorphic individuals with thin aortic walls. Perhaps that is a chance observation, but it has occurred sufficiently often so that I wonder if it has any significance.

(Dr. Jack D. Kirshbaum, Chicago, Ill.) Has Dr. McCartney seen any congenital malformations associated with the cases he has studied, because in several cases I have recently autopsied I did find some malformations which might suggest a congenital malformation of the media.

(Dr. McCartney, closing.) The records of these cases did not disclose anything particular in this group of 107 patients with regard to the body build. The whole group of these people varied from 13 to 94 years of age. There were some half-dozen of them where the dissection was apparently due to an acute aortitis, some following an acute upper respiratory infection. Most of them showed the medial necrosis that is ordinarily associated in the literature with a dissecting aneurysm. There were 3, or perhaps 4 cases, where there was a suggestion that syphilis might have been a responsible factor, although a syphilitic aorta is not supposed to split as readily as the normal aorta. There were in addition 3 or perhaps 4 definite cases of coarctation, of a certain degree. I do not believe that coarctation was diagnosed clinically in any instance. There was no other congenital anomaly.

THE INCIDENCE, ETIOLOGY AND FATE OF THROMBOSIS OF THE DEEP LEG VEINS IN 351 UNSELECTED AUTOPSIES. Warren C. Hunter, Portland, Ore.

Abstract. From four different hospitals, two charity and two private institutions, all possible soleus and gastrocnemius muscles from adolescent and adult bodies were routinely examined for phlebothrombosis. Among these were 185, or 52.7 per cent with proved ante mortem clots in different stages of evolution. Bilateral involvement was more frequent than unilateral; the veins of the soleus muscles were much more often the seat of thrombosis than were those of the gastrocnemius; the right side showed a greater frequency than the left, although the difference was not very striking. In the series there were almost twice as many males as females, and the disproportion between medical and surgical cases was even greater. However, in the available material there was no significant difference in the frequency with which the sexes were affected. Thrombosis occurred in 6.1 per cent more medical than surgical cases. The average age of those with thrombosis of the leg veins was 3.12 years older than those without thrombus formation. Thrombi were found in the youngest and the oldest of the entire group; the average age in the thrombus group was 59.78 years.

Correlation of clinical and pathological data points strongly toward (1) varying degrees of cardiac impairment, (2) cachectic states, and (3) confinement of older people to bed from any cause, without active exercise of the lower extremities, as the predisposing causes of phlebothrombosis in the legs. These factors were so often coexistent that it was often impossible accurately to evaluate their individual importance. Less often dehydration, abdominal distention and shallow breathing could be assigned a rôle. Atrophy of the muscle was found as often in individuals without as well as with thrombosis so that this appears to play no significant part in instituting clot formation. Phlebitis had a minor and insignificant part in causing thrombosis or even in accompanying the process. Only 14 veins displayed inflammation and in only 1 did this seem to be directly responsible for the thrombosis. In the rest, inflammation was so minimal that often only a segment of a vein wall proved to be inflamed. Hence it is felt that the almost universally used term thrombophlebitis is incorrect and misleading in so far as the deep veins of the calf are concerned. Phlebothrombosis is a better word for this situation. All thrombi could be classed as bland, the majority forming in dilated veins with a sluggish flow of blood, unaided by muscular action and often attached to vein valves, and all commonly showed evidence of propagation and healing. In several instances fresh thrombi were found in individuals who had been in bed for only 3 to 4 days. Most of them, however, formed in people dying on the 5th to 7th days after confinement to bed, and in another group those expiring between the 14th and 22nd days.

The fate of thrombi in the deep veins of the calf is not different from those elsewhere, save in one important particular, namely, more frequent fatal and non-fatal embolic episodes. Thus, 5 of the 11 cases terminating with fatal pulmonary embolism proved to have come from the veins of the legs alone. Furthermore, while 11.39 per cent of the entire group suffered non-fatal pulmonary embolism, the figure reached 20.5 per cent in those with thrombosis of the leg veins. Of these, a fair number sustained showers of emboli and lived long enough to develop pulmonary infarcts. All but 2 of the fatal cases were in men, but in the non-fatal group the sexes were equally affected. Aside from the embolic phenomena, it should be mentioned that the valves of the veins were often involved and with organization and canalization were destroyed and made functionless. Canalization in these veins is best developed peripherally in the form of often large, slit-like channels between the organized clot and the vessel wall.

Discussion

(Dr. Emmerich von Haam, Columbus, Ohio.) I should like to ask if Dr. Hunter has any idea of the age of the thrombi. Were they just ante mortem thrombi, or did they occur several weeks or days before the patient died?

(Dr. J. S. McCartney, Minneapolis, Minn.) I am very much interested in this paper of Dr. Hunter's. I have been working for some time on the question of thrombosis and embolism, particularly from the aspect of fatal pulmonary emboli, and have finally come to the conclusion that it is extremely difficult to distinguish between fatal and non-fatal pulmonary emboli, and it is also difficult to tell a pulmonary embolus from a pulmonary artery thrombus. I finally decided that the process should be looked at from

a little broader point of view, that we were really dealing with what might be called thromboembolic disease, putting together pulmonary embolism, infarction, pulmonary artery thrombosis and venous thrombosis. I have not quite completed a study of more than 25,000 autopsies done in a period of 20 years at Minnesota, and have found that there is an incidence of approximately 10 per cent in thromboembolic disease. This compares with Breslich's figures of 12 per cent and Belt's figures of about 11 per cent in a smaller number of cases. When we divided these autopsy figures into those with normal hearts and those with some gross changes in the hearts, in individuals with a sound circulation the incidence of thromboembolic disease is 5 per cent. Dividing the individuals with cardiac disease into those who had gross passive congestion of the liver and those without it, we find the incidence of thromboembolic disease in the cases of passive congestion jumps to 30 per cent, indicating that stasis is an extremely important factor in the development of thrombosis and resulting embolism. In the few instances in which I have been able to dissect the lower extremity extensively I have found thrombi in the situations which Dr. Hunter indicates. So far I have not been able to find any plantar vein thrombosis.

(Dr. Norbert Enzer, Milwaukee, Wis.) I should like to ask how often these emboli were multiple. Our experience indicates that in fatal instances multiple emboli occur more frequently than a single embolus.

(Dr. Hunter, closing.) In answer to Dr. von Haam's question as to the age of the thrombi, we tried to follow criteria which would hold water as to what constitutes a thrombus. When there was any doubt as to the nature of a clot it was our custom, because we were cross-sectioning the veins at intervals of 1 to 2 cm., to follow peripherally from the point where we chanced upon the clot, and it is a well known fact, observed by many, that the propagation of thrombi in veins is usually in the direction toward the heart and commonly we would find, even in freshly formed thrombi, at some point a difference that would enable us to say that some portion was probably older than another. Not infrequently this was at the site of a valve. Incidentally, our study showed that valves of the veins in thrombosis are ruined in about the same way that Edwards and Edwards showed in their experimental study a few years ago. The thrombi varied in age from those obviously quite fresh to those which were brown and in a state of softening, and to those which were fully canalized. In that particular group we looked back into the histories and often found that such patients had been in bed at some previous time for a matter of days or weeks. We found in these 185 cases thrombi of all ages from quite recent to very old. Rethrombosis of an old thrombus is not at all an infrequent occurrence.

I greatly appreciate Dr. McCartney's comment. We were already familiar with his previously published studies and are glad to hear that he now has a very large series on which to base conclusions. It is not often possible to examine the calf muscles and to remove them. One has to be unusually fortunate as regards the funeral directors to get their cooperation, and we were fortunate in that respect; otherwise we would have nothing to report. As concerns the plantar veins we felt we could not go too far, and we did not investigate these in a great number of cases. Our experience has been the same as Dr. McCartney's in that we have found plantar vein thrombosis very infrequent. We are trying to carry out that part of the study on amputated

legs and thus far, though the amount of the material is small, have been able to find thrombosis in very few of them.

In answer to Dr. Enzer's question as to how often multiple emboli are found, our experience is the same as his with respect to fatal emboli; they were not at all infrequent; often death was apparently the culmination of showers of emboli rather than as the result of a single embolus.

CHANGES IN THE JUXTAGLOMERULAR NEUROMYOARTERIAL APPARATUS (GOORMAGHTIGH) AND THEIR RELATION TO RENAL ISCHEMIA AND HYPERTENSION. Irving Graef, New York City.

Abstract. Since Ruyter's report (1925) of the presence of granular epithelioid cells in the media of the afferent arterioles close to the glomeruli of the mouse and rat, other observers (Oberling, Goormaghtigh, Zimmerman, Mathis, Clara, Becher, Elaut) have described the transformation of the fibrillar muscle cells to non-fibrillar "myo-epithelioid" cells in the walls of the afferent arterioles of various mammalian kidneys. The cytoplasm of these cells is described variously as sparsely granular (basophilic or acidophilic), clear or vacuolated. The special relation of this portion of the afferent arteriole to the segment of the distal convoluted tubule, which regularly makes contact with it, and the glomerulus was first observed by Zimmerman. He described a palisade arrangement of compact epithelial cells in the tubule at this area, which he designated as the "macula densa." He also introduced the term "Polkissen" to designate the pad of cells in the wall of the afferent arteriole where it touches the macula densa on the one hand and Bowman's capsule on the other. His findings have been confirmed by others. The transformation of myofibrillar cells to afibrillar cells in varying degrees backward in the arteriole and even in the interlobular arteries has been noted by some.

This preliminary report is a confirmation of the descriptions of the afferent arteriole, the macula densa and the appearance of the "Polkissen" in the mouse, rat, cat, rabbit, dog and man. The transformed cells are rarely granular, except in the mouse and the rat. Four presumably normal human kidneys have been studied. Three were fixed immediately after electrocution. The need for prompt fixation and differential staining to observe the changes is to be emphasized.

Recently Goormaghtigh and his associates have contended that this portion of the afferent arteriole undergoes hyperplasia, hypertrophy and metaplasia in renal ischemia and is the source of the pressor substance in renal hypertension. The special afibrillar cells have been likened to endocrine cells and to myoepithelioid cells in arteriovenous glomi. Hyperplastic changes have been found in the ischemic kidneys of 2 hypertensive human cases with unilateral arterial obstruction and in 1 case with pyelonephritis and unilateral perinephritis with marked contraction on the ischemic side. It was not observed in 2 non-hypertensive cases of pyelonephritis with focal scarring in 1 and severe scarring in the other.

In a surgically removed kidney from a non-hypertensive patient 4½ months pregnant, marked hyperplasia and hypertrophy were found. However, there was renal arteriosclerosis as well, with incomplete narrowing of the arterial lumens. Blocks from kidneys from other individuals dying during or at the end of pregnancy have been compared, and none showed similar hyperplasia.

Experimentally ischemic kidneys from dogs, rabbits and rats, with and without hypertension, have likewise been studied. Variation in the degree of hyperplasia has been observed in bilateral ischemia of severe grade. An additional finding has been the selective dilatation of the distal tubule in ischemic nephrons, with conspicuous dilatation at the level of the macula densa.

Studies in various nephropathies are in progress and will be reported later. Variation in the appearance of the arteriolar media in normal kidneys has been great enough to warrant doubt concerning the significance of the hyperplastic changes when observed without preliminary or concomitant evaluation of ischemia. Because of species differences the interpretation of the ischemic changes must be contingent upon a more complete study of normal kidneys, previous descriptions being based probably upon abnormal kidneys in which focal ischemia was not excluded. The relation of ischemia to hypertension has been established (Goldblatt) but the rôle of these changes awaits clarification.

Discussion

(Dr. Hans Popper, Chicago, Ill.) By means of fluorescence microscopy small, whitish fluorescing inclusions can be seen in the wall of the afferent arterioles in cases of hypertension. They are localized in places similar to those described by Dr. Graef, near the entrance of the afferent arterioles in the glomerulus close to the macula densa. These inclusions are not acetone-soluble; their fluorescence is not fading. In addition to hypertension, the inclusions were found in pregnancy. They were absent in cases of nephritis without hypertension.

THE PATHOLOGY OF ACUTE AND HEALED EXPERIMENTAL PYELONEPHRITIS. G. K. Mallory and (by invitation) A. R. Crane and J. E. Edwards, Boston, Mass.

Abstract. Unilateral pyelonephritis was produced by the intravenous injection of colon bacilli into rabbits that had a partial ligation of one ureter. By this means an acute pyelonephritis similar to that seen in man was produced in the obstructed kidney in about 75 per cent of the animals. An extensive pyelonephritis was never found in the unobstructed kidney. By releasing the ureteral obstruction at the end of 4 to 5 days after injection, a healing of the pyelonephritic process was induced. In this way stages of acute, healing and healed pyelonephritis were produced.

The acute lesions were found to arise (*a*) from interstitial abscesses starting around clumps of bacteria in the small blood vessels, and (*b*) as a result of organisms passing through the glomerulus into the tubules.

In the healed lesions thyroid-like areas of colloid casts were found. In earlier lesions a fusion of disintegrating polymorphonuclear leukocytes imprisoned in the tubules was found to lead to the formation of these casts.

The gross and histological appearance of the healed lesions confirms the criteria used by other authors and ourselves in the diagnosis of healed pyelonephritis in man.

Discussion

(Dr. Henry W. Ferris, Ithaca, N. Y.) As I understand it, the obstruction was removed in about 24 hours. I wonder if any investigation of the size of

the lumen of the ureter was made at the time the animals were sacrificed. The reason for this question is that a few years ago I was interested in some experiments with rabbits in which the ureter was partially occluded by a metal clip or slot in some rabbits, while in others it was completely tied off. In the first group after considerable time we saw the development of atrophic kidneys such as have been presented here. Presumably there was no infection; at least, we did not intentionally produce infection, and the results seemed to be due to partial obstruction of the ureter. I wondered whether the lumen of the ureter has been more or less permanently obstructed in these experiments.

(Dr. Mallory, closing.) I would like to correct one thing; we freed the obstruction at the end of 4 or 5 days, not at the end of 24 hours. After the freeing of the obstruction the lumen of the ureter returned to normal size. I know that these changes have been described as occurring in hydronephrosis. If my interpretation of their origin is correct, I do not see how they could occur without a superimposed secondary infection.

UROLITHIASIS MEDICAMENTOSA. Frank B. Cooper (by invitation), Paul Gross, and Ruth E. Scott (by invitation), Pittsburgh, Pa.

Abstract. The term *urolithiasis medicamentosa* is suggested as descriptive of the urolithiasis following the administration of certain of the sulfonamide drugs to date, such as sulfapyridine, sulfathiazole and sulfamethylthiazole. The incidence of sulfathiazole and sulfamethylthiazole urolithiasis in rats was less than that caused by sulfapyridine, and similar to the latter, was associated with secondary infection and pyelonephritis which occasionally caused death. Unlike the sulfapyridine uroliths, those caused by the thiazoles were more frequently found in the renal tubules and less frequently in the urinary passages. The uroliths were composed largely of the drug administered and were susceptible of spontaneous disappearance.

Discussion

(Dr. C. V. Weller, Ann Arbor, Mich.) Is there any discussion of this very timely paper?

(Dr. Esmond R. Long, Philadelphia, Pa.) I like the word "timely," and I think there is not only a time for the paper but a place for it. I attended a meeting of the Pathological Society of Philadelphia the other night and heard presented a case report of a similar picture in man. It seems to me that clinicians need more demonstration by pathologists of the danger of these drugs which are given in such huge amounts, and for that reason I should like to see such a paper presented before a clinical society and published in a clinical journal.

(Dr. Gross, closing.) I should like to add to Dr. Long's remarks that we have also seen a clinical case similar to that which Dr. Long mentioned; I believe he has in mind a case of which Dr. Pepper showed us a slide, and this slide, by the way, showed a striking similarity to the picture we produced experimentally. Our case, the report of which is in press at the present time, is one of gross hematuria due to sulfathiazol. The hematuria subsided with cessation of the drug.

CHRONIC NEPHRITIS OF OPOSSUMS (*Didelphys Virginiana*). Herbert L. Ratcliffe, Philadelphia, Pa.

Abstract. By far the most common type of renal disease of captive wild animals examined at the Philadelphia Zoological Garden is that which seems analogous to benign nephrosclerosis of man. Other lesions of the kidneys, particularly glomerulonephritis, rarely have developed. Of some interest, therefore, may be a form of subacute or chronic renal disease which has been observed thus far in 14 of 22 opossums from the colony maintained at the Morris Biological Farm of the Wistar Institute.

Two of the group were wild bred; the others had been reared in captivity. Ages have ranged from 11 to 33 months. Twelve were killed for examination because of injuries, failure to breed or general poor condition. Deaths of the remainder were attributed to infections, chiefly bronchopneumonia, to injuries by cage mates and, in one instance, to circulatory failure with ascites and hydrothorax. In 8 of the animals chronic ulceration of body surfaces was an associated lesion, and all have had some grade of dental caries or parodontitis. Neither cardiac hypertrophy nor arteriosclerosis have accompanied the disease of the kidneys.

The earlier microscopic changes were not always accompanied by definite alterations in the gross appearances of the organs, but, apparently, scarring may begin while the disease is still not far advanced. At this stage the capsular surface is marked by inconspicuous fine reticulations. In more advanced cases scarring is obvious, the surfaces being diffusely granular or dimpled by rather regular, small depressions, among which may be occasional larger scars.

Microscopic changes seem to center in and about the glomeruli and have been approximately equal in both organs. At first there is proliferation of the epithelium of Bowman's capsule or of the proximal tubule so that the glomerular space comes to be lined more or less completely by large cuboidal or columnar cells. Epithelium of the capillary tuft is increased at the same time and occasional cells are hypertrophied. The capillaries appear narrowed, occasional leukocytes accumulate in them, and endothelial nuclei are pyknotic. These alterations seem to be progressive. The epithelium forms adhesions with the tuft and capsule, Bowman's capsule and capillary walls are thickened, fibrous tissue increases about involved glomeruli, and eventually scarring is complete. When the disease is fairly advanced one finds all stages of its progress, some glomeruli enlarged and cellular, others small, relatively bloodless and with atrophic tubules surrounded by fibrous tissue.

Blood vessels have not shown significant changes, nor has there been evidence of active or healed pyelonephritis, but in the chronic stages of this disease perivascular infiltration of leukocytes frequently occurs. From the material that has been examined it seems that this disease may be regarded as a form of glomerulonephritis. Unfortunately there has not as yet been opportunity for study of the blood, urine and renal function, or of the bacteria involved in the dermal and oral lesions.

READ BY TITLE

PATHOLOGY OF THE LIVER FOLLOWING BURNS. L. J. Buis (by invitation) and F. W. Hartman, Detroit, Mich.

THE OCCURRENCE OF MICROBIAL INFECTIONS IN STRAINS OF CELLS MAINTAINED IN CONTINUOUS CULTURE AND THEIR TREATMENT WITH SULFANILAMIDE. George O. Gey, Baltimore, Md.

RHABDOMYOSARCOMATOUS PULMONARY METASTASES FROM A TERATOMA OF THE TESTIS. Milton D. Bosse (by invitation), Pittsburgh, Pa.

Abstract. Rhabdomyosarcomatous tumors of the testis or associated structures are uncommon. A case of teratoma of the testis which resulted in mixed metastases to the lungs and mediastinum is reported. The metastases contained large cells, some of which were elongated and showed readily demonstrable cross striations. Cartilage and considerable epithelial tissue were also present in the tumors. Inasmuch as skeletal muscle was not found in the primary tumor, the presence of a mixture of different tissues in many areas of the metastases suggested that the origin of the skeletal muscle metastases was from multipotential cells.

ADAMANTINOMA OF THE TIBIA. Horace B. Davidson (by invitation), Columbus, Ohio.

Abstract. Adamantinoma of the tibia is a locally malignant epithelial neoplasm arising in and remaining largely confined to the shaft of the tibia. The name arises from the histological and clinical similarities of this neoplasm to the well known tumor of the jaw. This tibial growth is comparatively uncommon, a total of 13 cases having been reported. The suggested etiology includes origin from traumatically displaced surface epithelium and from epithelial rests. The tumor occurs in young adult males and females, follows local trauma, has a long duration, and usually produces few symptoms. Radical resection or amputation is usually necessary for cure. The tumor is locally invasive but does not metastasize. The X-ray plate is apt to show multilocular cystic destruction. The histology is characterized by basal epithelial cells usually arranged in solid cords but sometimes forming cysts or epithelial pearls.

The case reported appears to be the 14th described, and concerns a 26 year old white male. He complained of swelling in the lower left leg, and pain in this region on walking. Twelve, 10 and 13 years before, this portion of this leg had been fractured. Following the last fracture, what was thought to be a bone cyst was removed from the left tibia. X-ray examination showed rounded areas of rarefaction in the middle of the left tibia. The diseased bone was removed and a diagnosis of cyst of the bone was made. Histological examination showed solid cords of well differentiated basal and prickle epithelial cells infiltrating through a dense stroma. Other primary sites of malignancy were eliminated clinically. One and one-half years after the operation the patient is walking and working, but has a persistent draining sinus, and complains of periodic pain.

RHINOSPORIDIOSIS. Horace B. Davidson (by invitation), Columbus, Ohio.

Abstract. Rhinosporidiosis is a chronic productive inflammatory lesion caused by the *Rhinosporidium seeberi* and commonly attacking the nasal mucosa of young males. The first case was reported by Seeber from Argentina in 1900. Since then cases have been reported from India, Ceylon, the Phillipine Islands, Africa, Europe, South America and North America. Eight cases have been reported from North America. The organism has been classed as a lower fungus. Its life cycle begins with a single cell, the nucleus of which divides repeatedly, forming a large sporangium with a chitinoid membrane which ruptures and discharges spores which initiate a new cycle. The mode of dissemination of the organism has not been determined. Human cases are usually found in boys or young men. Most cases show involvement of the nose, although other anatomical sites are mentioned. The morbid anatomy is characterized by the formation of a polyp. The histopathology presents three outstanding features: the *Rhinosporidia* are found in all stages of development; immediately around a recently ruptured sporangium there is found a small abscess; the bulk of the polyp is composed of granulation tissue. The clinical picture is dominated by progressive nasal obstruction, nasal discharge and nasal bleeding.

The case here presented is the 9th reported in this country and occurred in a 6½ year old boy. The clinical duration was only 3 weeks and the only symptom was nasal obstruction. Three months after removal of the polyp there has been no recurrence. This case differs from the others in that the patient was younger, the duration shorter and the symptoms fewer.

AMEBIASIS CUTIS: REPORT OF A CASE. Bromley S. Freeman (by invitation), Robert Schrek and (by invitation) Paul F. Brown, Hines, Ill.

Abstract. A case is reported of a 48 year old laborer with a history of recurrent dysentery for 20 years. Two years prior to admission a progressive swelling and ulceration of the abdominal wall developed after a bruise with a dirty shovel. Surgical and medical treatment at various clinics did not prevail against the lesion. The patient was sent to the tumor clinic with a diagnosis of carcinoma of the abdominal wall.

Physical examination showed an emaciated male with a foul-smelling abdominal ulcer, measuring 15 cm. in diameter, and 3.5 cm. deep. The base of the ulcer appeared to be on the peritoneum. A sinus was injected with lipiodol but revealed only a long sinus in the lateral abdominal muscles and no fistula could be demonstrated. A biopsy revealed necrotic tissue and numerous amebae in the adjacent viable tissue. The amebae contained phagocytized red blood cells, were motile in fresh smears, and could be cultivated on special mediums. The parasites were identified as *Entameba histolytica.* Similar amebae were obtained from the feces. Bacteriological cultures of the ulcer revealed numerous types of organisms, including amebic non-hemolytic streptococci.

Both the general condition and the ulcer improved greatly following a course of emetine and daily zinc peroxide dressings. The wound healed completely without the use of skin grafts.

THE CONCEPT OF FETAL ENDOCARDITIS. Paul Gross, Pittsburgh, Pa.

Abstract. The term fetal endocarditis or endomyocarditis has been applied to more than 50 cases reported in the literature. The criteria employed in making this diagnosis have been varied and loose. About one-third of the reported cases were not examined microscopically and in one-half of the cases no cellular infiltration was noted. Although more than one-half of the cases showed valvular deformities, the absence microscopically of cellular infiltrates or other indications of inflammation gave no support to the assumption of a valvulitis.

Myocardial scars, stressed as a prerequisite for the diagnosis of fetal endocarditis, have been noted in cardiac anomalies generally accepted to be developmental defects. The assumed etiological pathogen in the cases of so-called fetal endocarditis has been either anamnestically absent or variable in identity.

A cardiac anomaly in a newborn infant is reported which satisfies most of the criteria of so-called fetal endocarditis. Microscopic sections of nodular and adherent aortic cusps, as well as of a funnel-shaped mitral valve with thick, retracted chordae tendineae show normal immature connective tissue without scarring, cellular infiltration or excessive vascularization. Myocardial scarring with focal calcification present in a papillary muscle resembles bland infarction rather than inflammation.

An associated, diffuse and uniform endocardial fibrosis of the diminutive left ventricle similarly shows no remnants of an inflammatory process. This diffuse parietal endocardial sclerosis has obliterated the thebesian and arteriolumina vessels. Although it is not known what rôle these vessels play in the nutrition of the fetal myocardium, it is possible that their obliteration may be related to the myocardial scarring. The overgrowth of the parietal endocardium is considered to be a developmental defect, similar to the diminutive size of the left ventricle and the valvular deformation.

THE CELLULAR STRUCTURE OF CARCINOMA OF THE LUNG. A STUDY OF NINETY-TWO CASES. Béla Halpert and (by invitation) Bjarne Pearson, New Orleans, La.

Abstract. This study of the microscopic structure of carcinoma of the lung was undertaken to assemble data concerning the cellular origin of these growths. Among the 92 cases analyzed, 49 (over 50 per cent) were squamous cell, 17 (approximately 20 per cent) were columnar cell, and 26 (approximately 30 per cent) were reserve cell carcinomas.

The growths were classified as reserve cell carcinomas when the tumor cells were of the same size, the nuclei round, oval or elongated and deeply staining, the cytoplasm scanty, and the cell borders hardly discernible. The cellular arrangement formed no particular pattern; in some growths the cells were arranged in whorls, in others there was a palisade arrangement of the peripheral cells.

The occurrence of growths composed entirely of reserve cells, the demonstrable transformation of reserve cells into either squamous cells or columnar cells, and the occurrence of carcinomas composed of all three types of cells seem to support the concept that the reserve cell is the parent cell of all carcinomas of the lung.

MULTIPLE MYELOMA WITH METASTASES TO THE TESTICLES AND THE PANCREAS. Jack D. Kirshbaum, Chicago, Ill.

Abstract. Sixteen cases of myeloma were selected from 13,000 consecutive autopsies (1929 to 1940) for this study. Histologically the cases were all of the plasmocytoma type. There were 11 males and 5 females; of these, 10 were white and 6 colored. The ages of this group were as follows:

2	between	35	and	40	years
5	"	41	"	50	"
4	"	51	"	60	"
5	"	61	"	70	"

The distribution of the lesion in the 16 cases to the various bones included: vertebra 12, ribs 11, skull 8, sternum 5, clavicle 3, pelvis 3 and femur 2. In 5 cases the neurological symptoms predominated the clinical picture. This was due to extension of the lesion into the vertebral canal and compression of the spinal cord. The most remarkable feature of the plasmocytoma in this series of cases was the metastases to the internal organs in 2 cases, pancreas, liver and right adrenal, and pancreas and both testicles respectively. This report is concerned with the unusual site of involvement, namely both testicles. Only two previous papers in the literature have ever mentioned the presence of multiple myeloma in the testicle.

The case to be reported was a 62 year old white male who was admitted to the Neurological Ward with paraplegia and urinary retention. He had been ill for 6 weeks and suddenly became paralyzed a week before admission. The clinical diagnosis was transverse myelitis with a cord tumor.

The essential autopsy findings were a plasmocytoma of the fifth thoracic vertebra with extension into the paravertebral tissues and vertebral canal; compression of the spinal cord and myelomalacia; metastasis to the body of the pancreas and to both testicles, more marked in the left; suppurative prostatitis and cystitis; and bilateral pyelonephritis.

Histologically the cells in the vertebra and pancreas were typical of the plasma cell type, whereas in the testicle the picture was pleomorphic. There were many plasma cells and large numbers of cells with an irregular, finely trabeculated nucleus and an ample, pink staining cytoplasm. Some of the nuclei were multinucleated.

CONGENITAL BICUSPID AORTIC VALVES. Simon Koletsky (by invitation), Cleveland, Ohio.

Abstract. In 3300 consecutive autopsies a congenital bicuspid aortic valve was found in 18 cases (5.4 per cent) and was the most common single cardiac anomaly. Nine cases occurred in infancy and childhood and 9 in adult life; 14 were in males and 4 were in females. Next in order of incidence were coarctation of the aorta (16 cases) and patent interventricular septum (14 cases). The most frequent associated cardioaortic anomalies were coarctation of the aorta, patent interventricular septum and patent ductus arteriosus, but in 9 of the 18 cases the bicuspid aortic valve was the only congenital deformity in this region.

Seven cases showed simple bicuspid valves, but in 11 cases there was congenital fusion of two cusps, the resultant ridge being at commissure A in 6

cases, at commissure B in 3, and at both commissures in 2 cases; no fusion was found at commissure C.

The gross character of the congenital ridge is so distinctive as to be practically pathognomonic. It consists of a narrow, hemicylindrical, bar-like elevation situated at the commissure, directed in the long axis of the aorta and extending slightly and uniformly into the sinus of Valsalva. Microscopically the ridge consists largely of elastica, whorled centrally and continuous laterally with that of the aortic media. The elastica usually overlaps the annulus fibrosus both superficially and deeply, but may overlap it only superficially or deeply.

The commissural raphe of an acquired bicuspid aortic valve differs in that it is constituted largely of relatively acellular collagenous tissue, has little or no elastica, and shows the presence of residua of other disease.

Superimposed disease of the aortic valve was not found in any of the cases in early life; but in the form of rheumatic involvement, endocarditis lenta or calcified sclerosis was present in 7 of the 9 adults. The congenital ridge is affected by the superimposed disease only rarely. The study suggests that this congenital anomaly may predispose to calcific sclerosis.

MALIGNANT MELANOMA. Shields Warren and Olive Gates, Boston, Mass.

Abstract. While malignant melanoma is frequently assumed to be a hopeless therapeutic problem it has but little worse prognosis than many other malignant tumors. Thus the 3 year cures in our series of 172 cases of malignant melanoma from the Huntington Hospital, Pondville Hospital and the Tumor Diagnosis Service, total 34 per cent, as compared with 29 per cent for cases of fibrosarcoma from the same sources. Our figures for 5 year cures are 20 per cent as compared with 24 per cent for Grade II and III epidermoid carcinoma of the skin.

Attempts at histological grading have not been successfully correlated with group prognosis. The size of the lesion when first seen does not appear to be a factor of prognostic value.

In differentiation of malignant melanoma from pigmented nevi it must be remembered that some nevi show mitotic activity. Invasion is difficult to determine, and many nevi appear to invade either the epidermis or the corium. We have found abnormally large or distorted cells the best criterion of malignancy.